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KENNETH B. RAPER, PRESIDENT, 1951

# MYCOLOGIA

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VOL. XLIV JANUARY-FEBRUARY, 1952 No. 1

## A DECADE OF ANTIBIOTICS IN AMERICA<sup>1</sup>

KENNETH B. RAPER

(WITH PORTRAIT AND 4 FIGURES)

### INTRODUCTION

In the summer of 1941 Professor H. W. Florey and Dr. N. G. Heatley of Oxford University brought to America the problem of producing penicillin, thereby setting in motion a most remarkable chain of events. An unparalleled interest has developed in all groups of microorganisms and in the products of their metabolism. A multimillion-dollar antibiotics industry providing employment for thousands has been securely established. Medical practice has been profoundly influenced, and cures then unknown are now accepted as routine. Unmeasurable suffering has been alleviated, and the lives of countless individuals have been saved. All these things are known to you. Yet, I think it appropriate, on this tenth anniversary of their mission, to recall some of the epoch-making discoveries and far-reaching developments which have marked this "Decade of Antibiotics."

Important as are the developments since 1941, man's awareness of microbial antagonisms reaches back much further. There is a comparatively rich folklore from many nations suggesting the use of moldy bread and similar products to combat "gatherings" and other superficial infections. Dating back to Pasteur, scientific publications are not infrequent wherein a benign microorganism was observed to inhibit or even destroy a highly infectious one.

<sup>1</sup> Presidential Address, Mycological Society of America, 1951. Presented in abbreviated form, September 10, 1951, at the Society meetings in St. Paul, Minnesota.

[*MYCOLOGIA* for November-December (43: 605-744) was issued January 15, 1952]

Particularly prominent among such report are references to the properties and use of pyocyanase, popularized by Emmerich and Low (1899, *et seq.*). This background of ante-penicillin work and publication has been carefully and interestingly reviewed by Prof. Florey as the first chapter in the monumental work on antibiotics brought out by his Oxford team in 1949.

#### PENICILLIN

Interest in microbial antagonists seemingly subsided during the 1920's, and there is little to indicate that Fleming was influenced appreciably by this earlier work. Thus, in all truthfulness, the modern age of antibiotics can be dated from his discovery of penicillin in 1928, which was announced the following year in a paper beginning with this now famous paragraph:

"While working with staphylococcus variants a number of culture-plates were set aside on the laboratory bench and examined from time to time. In the examinations these plates were necessarily exposed to the air and they became contaminated with various microorganisms. It was noticed that around a large colony of a contaminating mould the staphylococcus colonies became transparent and were obviously undergoing lysis."

The rest of this story, often retold, is familiar to all of you. Less well known is the fact that little study was given to penicillin during the following decade, although Fleming had established the microbial spectrum of his antibacterial substance with surprising accuracy and had called attention to its potential therapeutic importance. Relative to the latter, he commented as follows:

"Penicillin, in regard to infections with sensitive microbes, appears to have some advantages over the well-known chemical antiseptics. A good sample will completely inhibit staphylococci, *Streptococcus pyogenes* and pneumococcus in a dilution of 1 in 800 . . . which is more than can be said of the chemical antiseptics in use. Experiments in connection with its value in the treatment of pyogenic infections are in progress."

Professor Raistrick and co-workers (Clutterbuck *et al.*, 1932) subsequently confirmed the antibacterial properties of Fleming's penicillin, and in addition gained the first insight into its chemical insta-

bility in acid and alkaline solutions. Looking back, it is interesting to speculate how our story might have developed had he succeeded in interesting his physician friends in testing penicillin when and if an adequate amount could be produced. In this country, Roger Reid (1933, *et seq.*) substantiated the results already recorded. Bornstein (1940) used penicillin (culture filtrates) as an aid in isolating enterococci and streptococci. That is all—and, as Coghill has pointed out (1944), penicillin nearly died a natural death.

Although the Decade of Antibiotics which I shall now discuss begins chronologically in 1941, the actual development began two years earlier in the William Dunn School of Pathology, Oxford University, when Florey, E. Chain, and Heatley undertook an investigation of antibacterial substances produced by microorganisms. Penicillin and Fleming's mold, then correctly identified by Thom as *Penicillium notatum* Westling, commanded their attention from the beginning. A limited amount of the active substance was produced, additional information on chemical characteristics was obtained, and most important of all, some *in vivo* tests in infected mice were performed with singularly favorable results. A brief report (Chain *et al.*) appeared in the *Lancet* in August 1940. The following year, a longer and more significant paper (Abraham *et al.*) was published in the same journal. Methods for producing penicillin in earthenware vessels on a comparatively large scale (1941 'standards!') were described; the cylinder plate assay technique was introduced and the penicillin *unit* defined; attention was given to the effect of penicillin on bacteria and animal cells; further animal experimentation was performed; and, most important, the first clinical trials were made with amazingly auspicious results. Prof. Florey (1949, Chapter 15) has written a full account of this early work, and regarding a problem in production he tells the following story:

"It may be amusing to recount the evolution of the first vessels. Apart from the usual laboratory ware, trials were made with various kinds of glass and enamel domestic dishes and utensils, and biscuit and other tins (both with and without a coating of lacquer or varnish), but it was found that the old-style bedpan with a side-arm and lid was an ideal culture vessel, providing a relatively large surface area over a shallow layer of fluid and with a side-arm for

inoculation and withdrawal. Unfortunately when an effort was made to procure 600 of these vessels it was found that such a large number could not be provided as they had been replaced by a more modern streamlined structure without a lid. At the time they were required the Battle of Britain had been won but the country was being subjected to heavy bombing so that it was difficult to secure supplies of any sort. Glass vessels could not be made within a reasonable time, but Messrs. J. Macintyre & Co., of the Staffordshire pottery industry, undertook to make special rectangular porcelain vessels, fitted with a side-arm, which could be readily stacked in incubator and sterilizer. To overcome transport difficulties Heatley borrowed a van and drove 200 miles to fetch the first consignment. He returned with them in a snow-storm on 23 December and they were first sown with *Penicillium notatum* on Christmas Day, 1940."

Due to the serious war situation in England during the summer of 1941, it was deemed inadvisable to attempt there the production of additional penicillin needed for extensive clinical trials. For that reason, and aided by the Rockefeller Foundation, Florey and Heatley came to the United States. Here they were referred to the National Academy of Sciences and to Dr. Charles Thom, principal microbiologist in the Department of Agriculture in Washington. They were advised to come to the Northern Regional Research Laboratory in Peoria where the staff of the Fermentation Division had had experience in mold fermentations and where a large collection of molds was maintained. Dr. O. E. May, Director of the Laboratory, and Dr. R. D. Coghill, head of the Fermentation Division, realized the potentialities of the drug, and arrangements were made to begin work on the problem immediately. Thus was initiated in America the main stream of research in antibiotics.

Before pursuing further our discussion of penicillin research, let us take cognizance of some important developments taking place in other fields and in other laboratories. In California, Richard Weindling had shown (1934) that the common soil fungus *Gliocladium fimbriatum* produces a substance, "gliotoxin," capable of destroying the hyphae of *Rhizoctonia solani* and other fungi. Two years later, in collaboration with O. H. Emerson, he isolated the substance in crystalline form, thus becoming the first to isolate in a pure state a substance known to have antibiotic properties. At

the Rockefeller Institute, René Dubos (1939, *et seq.*) had demonstrated that a common soil bacillus, subsequently identified as *B. brevis*, was capable of producing a bacteriocidal agent highly active against certain virulent types of pneumococci. With the collaboration of Hotchkiss and others, he had shown that this agent, designated tyrothricin, consisted of two active principles, gramicidin and tyrocidine, and suggested that the former might find application in the treatment of infections caused by gram-positive cocci. At Rutgers University, Professor Waksman was actively investigating the antagonistic effects of various soil microorganisms—investigations which were to culminate three years later in the discovery of streptomycin. Whereas virtually nothing had been done with Fleming's mold in America, many investigators, conditioned by reports then issuing from the above laboratories, were keenly aware of the possibilities for research in the field of microbial antagonism. A fertile seed bed ready for planting awaited Florey and Heatley when they arrived in this country.

At the Peoria Laboratory it was decided that the penicillin problem should be investigated from three different angles. First, we would attempt to improve penicillin yields by studying the nutrition of the mold in surface cultures of the type then employed in England. Second, we would endeavor to develop a method for producing the antibiotic in submerged culture. Third, we would investigate other molds in the hope of finding a more productive strain. It is a matter of record, and a source of pride to all of us that we were, in time, able to realize all of these objectives.

The nutrient solution was altered in many ways by Dr. A. J. Moyer, microbiologist, and many substances known to promote the growth of molds were investigated for their ability to increase penicillin formation. Of such substances, corn steep liquor was found to be outstanding. Different carbohydrate sources were likewise investigated, and lactose was found to be most favorable. The Laboratory therefore recommended for penicillin production a nutrient solution whose principal ingredients were lactose and corn steep liquor (Moyer and Coghill, 1946a), and with certain modifications, depending upon the mold culture and the method of production employed, this medium is still in general use.

Earlier successes with the gluconic, lactic, and fumaric acid mold fermentations suggested the probability that penicillin could be produced in submerged culture—a technique introduced first by Kluyver and Perquin in 1933. Attention was early directed toward this objective, and different molds in our Collection that belonged to the *Penicillium notatum-chrysogenum* group were investigated. A strain of *P. notatum* (NRRL 832), not genetically related to the Fleming isolate, was found to produce promising yields. Although more productive strains have long since supplanted it, this culture was used for the first commercial production of penicillin by the submerged culture, or deep tank, process of manufacture. A nutrient solution was employed that contained approximately  $\frac{1}{2}$  the concentrations of corn steep liquor and lactose recommended for surface culture (Moyer and Coghill, 1946b).

It was realized from the outset that if penicillin could be produced in submerged culture, this would represent the best means of achieving large-scale production at moderate cost. For example, assuming equal yields (per ml.) by submerged and surface culture methods, it was calculated that a single 10,000-gallon tank would represent the equivalent of 60,000 to 70,000 two-quart bottles in production capacity and would be much less expensive to operate. By the beginning of 1944 a substantial part of the U. S. production stemmed from this source and by the end of 1945 it represented the total amount. The immediate effect of this development was to produce far greater quantities of penicillin than could have been possible otherwise; the longer-range effect was to obviate the necessity of time-consuming studies with surface cultures when developing subsequent fermentations such as streptomycin, chloramphenicol (Chloromycetin), aureomycin, and terramycin. In current antibiotics research even the primary screening is conducted in submerged (shaken) cultures.

We should not, however, dismiss other methods of production without recognizing the contribution that these made. All of the early work in England was done with surface cultures. More important, it was with penicillin from surface cultures that the efficacy and applications of the drug were definitely established. Much credit must go to those industrial firms, who, believing that

submerged production would represent a more profitable ultimate goal, nevertheless constructed expensive bottle plants in order that the armed services and the civilian population might have penicillin more quickly. Some penicillin was produced by modifications of the so-called mold bran process—in one case growing the mold on sterilized bran in shallow pans, in another case on bran rotated in circular drums. One investigator suggested using a chip-packed tower simulating a vinegar generator. Several groups produced "crude penicillin" by growing the mold directly on sterile gauze bandages saturated with a suitable nutrient solution. In Hawaii, during World War II, Carpenter and co-workers (1945) produced such preparations in considerable number for use in the Islands and by the U. S. Navy in the South Pacific area. It seemed that everyone wanted to be in on the fun, and the Sunday editions of many metropolitan papers ran feature stories telling "how to make penicillin in the kitchen." Coghill and I (1943) felt constrained to warn of dangers inherent in such practices carried out in the absence of rigorous asepsis.

Much attention was given to strain selection and improvement, and singular success attended such efforts all along that line. Fleming in his first report referred to his mold as *Penicillium rubrum* Biourge. Raistrick subsequently submitted it to Thom who identified it as *P. notatum* Westling. The importance of Thom's corrected diagnosis is obvious since it identified the culture with a cosmopolitan series of molds and in later years gave direction to the intensive search for more and better penicillin-producing strains. The early work in America, as in England, was done with the original and unaltered Fleming strain. Yields up to 75 to 100  $\mu$ /ml. were realized in surface cultures with the improved lactose-steep liquor medium, in contrast to the 2 to 4  $\mu$ /ml. yields obtained when the work began. From single spore isolations and tests of the resulting clones, a substrain, NRRL 1249.B21, was obtained which produced yields up to 175 to 200  $\mu$ /ml. This substrain was much used for the surface culture production of penicillin in this and allied countries so long as this method of manufacture was employed. Attempts to further improve production by the selection of natural variants were unsuccessful. The

same technique was applied to strain NRRL 832 but none of the variants produced penicillin in significantly greater amounts than the parent. Attention was then directed toward the isolation of new strains from nature, for work already done had demonstrated that almost all members of the *P. notatum-chrysogenum* group would produce *some* penicillin. It seemed reasonable to expect that if sufficient new isolates were examined a markedly superior strain might be discovered. Isolations were made from moldy food products, fruits and vegetables, and from a great variety of soils collected from stations in the United States and from many foreign countries. In this search we had the active cooperation of the Army Transport Command. Penicillin, by this time, had seized the public imagination, and many individuals, wishing to contribute their bit, sent materials to us for examination. I recall particularly an Arizona rancher who dispatched a lichen-encrusted rock ("covered with green mold"), adding that he could send us tons of like material if this would speed the investigation.

Interestingly enough, the most important culture discovered was isolated from a moldy cantaloupe collected in Peoria. This strain of *P. chrysogenum* Thom was designated NRRL 1951, and in the months that followed it became the object of intensive study leading to the development of increasingly more productive strains upon which all subsequent manufacture of the drug was based. By successive single spore isolations a substrain, NRRL 1951.B25, was obtained which increased yields from about 70 to 80  $\mu$ /ml. (NRRL 1951) to 200 to 250  $\mu$ /ml. Repeated attempts at further improvement by selection of natural variants were unsuccessful. At this stage the Office of Production Research and Development set up projects at several laboratories to discover or develop more productive cultures. The principal outcome of this work up to 1946 has been reported as follows:

"At the Carnegie Institution a mutation was produced that possessed outstanding merit. This culture, designated X-1612, was produced by X-ray radiation of spores of NRRL 1951.B25. It was first tested at the University of Minnesota, but its real potentialities were established at the University of Wisconsin in small vat fermenters. . . . Yields more than twice those produced by NRRL 1951.B25 were obtained from X-1612 and it soon sup-

planted the parent culture as the principal strain for commercial production. Another great step forward was made by exposing spores of *X-1612* to ultraviolet. In this way, the Wisconsin group succeeded in producing a mutation, designated *Q-176*, which doubled the yield produced by strain *X-1612*. The development of this outstanding culture for submerged production can be summarized as follows:

NRRL 1951	<i>P. chrysogenum</i> , isolated from a moldy cantaloupe, capable of producing approximately 100 $\mu$ /ml. of penicillin in submerged culture.
NRRL 1951.B25	A naturally occurring variant from NRRL 1951, capable of producing up to 250 $\mu$ /ml. of penicillin.
<i>X-1612</i>	An X-ray-induced mutation from NRRL 1951.B25, capable of producing more than 500 $\mu$ /ml. of penicillin.
Wis. <i>Q-176</i>	An ultraviolet-induced mutation from <i>X-1612</i> , capable of producing more than 900 $\mu$ /ml. of penicillin" (Raper, 1947).

Subsequent to these developments, and continuing up to the present, Professors Backus and Stauffer (1946, *et seq.*) have made an intensive study of strain variation and mutability in Wis. *Q-176* and its derivatives. By the reapplication of ultraviolet radiation and exposure to nitrogen mustards, combined with careful selection, they have succeeded in isolating a series of high-yielding, pigmentless strains culminating in strain Wis. 49-2105 which is capable of yielding 1500  $\mu$ /ml. in shaken flasks and in 30-liter stirred jars. In discussing Wis. *Q-176* and the pigmentless forms derived from it, Backus has pointed out that these "strains" in reality consist of a mixture of cultural types which are generally present in fairly constant proportions with the "U," or usual, type predominant. He writes:

"Thus our strains are not homogeneous entities but, rather, conglomerates with a pattern. However, most of the strains can be successfully propagated through the usual mass-transfer techniques, apparently because the predominant type is also the most vigorous and in addition produces the various types of spores in a proportion approximating that in the original stock. If a plate

culture is started from a mixed spore inoculum or from a mass of hyphae, you will obtain a colony of the predominant type only, for the other elements will have no chance to show up in the competition. Still they are basic components of the race and may readily be separated out." (personal correspondence)

From the work in our Laboratory and at Wisconsin certain significant points emerge. First, molds belonging to the *P. notatum-chrysogenum* series are generally characterized by great natural variability. Second, in respect to penicillin production, limited improvement can be expected by taking advantage of such inherent variation. However, for each strain there seems to exist an upper limit beyond which this phenomenon cannot be exploited. This has been demonstrated in the Fleming strain, NRRL 832, NRRL 1951, and other primary isolates not cited here (Raper and Alexander, 1945). Third, by application of mutagenic stimuli variability can be tremendously enhanced, including marked improvements in biosynthetic capacity. Fourth, mutants obtained by such techniques do not represent uniform clones, but continue to exhibit extensive variability within themselves. Once, when talking with a geneticist, I inquired as to the basis for such behavior, and I was told that the cultures were obviously heterozygous. Being a non-geneticist, I hope you will pardon me if I inquire: How did an apparently sexless mold such as *P. chrysogenum* acquire this heterozygosity, and how much of it can be contained in a single uninucleate spore? Strain Wis. 49-2105 is, I am sure, the result of scores, if not hundreds, of single spore isolations in a linear series. I should think that the original instability of NRRL 1951 might have become diluted to negligibility long before this.

Penicillin as it is produced by mold cultures does not represent a discrete chemical compound but rather a family of closely related compounds which have a common lactam-thiazolidine structure with differing side chains, or R groups. Today pharmaceutical penicillin is entirely penicillin G (more specifically benzylpenicillin). This is dictated by certain circumstances. Penicillin G was the first penicillin to be isolated in appreciable quantity; it possesses highly desirable characteristics from the standpoint of recovery and purification; and the strain improvement program (coupled with supplemental feeding of appropriate precursors) has been consist-

ently directed toward its elaboration. Penicillin K, or heptylpenicillin, does not constitute a suitable drug since adequate blood levels cannot be maintained in the body. Penicillin X, or p-hydroxybenzylpenicillin, on the other hand, is quite acceptable from this standpoint and on a per unit basis is more effective against streptococci, pneumococci and gonococci than penicillin G. However, yields tend to be low and only limited attempts have been made to increase productivity (Raper and Fennell, 1946). Penicillin F, or  $\Delta^2$  pentenylpenicillin, and dihydropenicillin F, or amylpenicillin, have never been available in sufficient quantities for clinical evaluation. Differences between the antibiotic activities of all the natural penicillins is largely quantitative rather than qualitative, and organisms insensitive to one are generally insensitive to the others. At the Northern Laboratory, Stodola prepared numerous chemical modifications of natural penicillins; and at the Lilly Laboratories, Behrens produced biosynthetically a large number of penicillins by supplementing the culture broth with suitable precursors. This work was published in "The Chemistry of Penicillin" (1949) as Chapters XX and XIX respectively. In general, these behave as the natural penicillins. It is interesting to note that mold strains *X-1612* and *Wis. Q-176* naturally produce a high proportion of penicillin K; however, by using suitable adjuvants such as phenylacetic acid in the nutrient solutions, high yields of penicillin G are regularly obtained. This practice has been used in industry for several years.

Ordinarily we think of penicillin as a metabolic product characteristic of the *P. notatum-chrysogenum* series. However, Florey *et al.* (1949) have listed as penicillin producers 7 species of *Aspergillus* and 12 species of *Penicillium* outside this series. From an entirely different group of fungi, Peck and Hewitt (1945) reported the production of a penicillin-like antibiotic by *Trichophyton mentagrophytes*.<sup>2</sup> In the face of these developments mycologists have had to alter radically their views as to the specificity of metabolic products. Penicillin was first regarded as a unique product of Fleming's isolate, then of the general series to which it belonged, and now it is recognized as common to many groups. Clavacin

<sup>2</sup> It is significant that some clinicians attribute penicillin sensitivity in patients to prior histories of *Trichophyton* infections.

(= patulin, penicidin, claviformin, clavatin, and expansine), and gliotoxin, to cite two additional antibiotics, are likewise known to represent metabolic products of diverse fungi. Citrinin, first isolated from *Penicillium citrinum*, is even produced by the legume *Crotalaria crispata* (Ewart, 1933). Gone are the days when a biochemist, or microbiologist, could identify a product and thereby assume that he had identified an organism!

By the summer of 1951 penicillin production in the United States had reached 25 to 30 trillion units per month. The greatest single factor contributing to this phenomenal development is undoubtedly the introduction of increasingly productive mold strains, and in this accomplishment all mycologists can take justifiable pride. Furthermore, these improved microbial tools are being used with ever increasing effectiveness. As fermentologists have gained greater know-how in conducting mold fermentations, this experience has been reflected in improved production and processing techniques. Development of the pigmentless strains has led to more efficient use of precursors and to economies in drug recovery and purification. It is particularly noteworthy that the production of penicillin in submerged culture still rests solidly upon the principles established more than eight years ago, and the basic nutrient solution then recommended is still employed without substantial modification. However, much has been learned in the meantime. Regimens for developing inocula have been improved; critical studies relative to aeration and agitation have been made; and a reasonably complete picture of the chemical changes which take place during the fermentation has been obtained. Pioneer work was done in our Laboratory along all of these lines, and this was paralleled in the research laboratories of those manufacturers who early undertook the production of penicillin in deep tanks. Professors W. H. Peterson, Marvin Johnson, and co-workers at the University of Wisconsin have investigated all aspects of this fermentation, and the extensive series of papers issuing from their laboratory constitutes the most complete documentary reports published on this subject.

In an address entitled, "Recent Advances in the Penicillin Fermentation," presented in Rome in June, 1951, Johnson stresses certain factors contributory to high penicillin yields. Adequate

effective aeration must be provided, and this concerns the amount of oxygen available to the mold rather than the amount passing through the fermenter. Dispersion of air by vigorous agitation is imperative. Adequate but non-toxic levels of precursor must be available during penicillin biosynthesis, and this is best realized by intermittent feeding. Since pharmaceutical penicillin is penicillin G, phenylacetic acid or some derivative is the preferred precursor. Adequate mold growth is a prerequisite for penicillin formation, and this is best obtained in an acid medium pH 4.5-6.0 with readily available nutrients such as ammonium ions and glucose. Having obtained the necessary mycelium, the pH is raised to 7.0 or above and a slowly assimilable carbohydrate (e.g., lactose) is substituted, after which growth is minimal and penicillin formation proceeds at a rapid rate.

Penicillin yields in excess of 1500  $\mu$ /ml. are apparently not uncommon today. Stock cultures employed for this production are generally conserved in soil. Not infrequently reisolation of single colony clones is undertaken to maintain or reestablish high productivity. Beyond this point the production culture is handled by infrequent mass transfer of spores. A minimum number of culture generations is used to secure an inoculum of young, vigorously growing mycelium adequate for seeding the large fermenters, and the mold is not permitted to sporulate during this inoculum build-up.

Fermentations are carried out in tanks of varying size up to 30,000 gallons, and too high praise cannot be given to the engineers and microbiologists who designed this equipment and learned to sterilize vast amounts of air so that it can be operated under completely aseptic conditions.

After 10 years, penicillin still remains the least toxic and the most generally useful of all the antibiotic drugs. During recent years its range of applicability has been extended, but the most outstanding development has been its ever-widening use as a prophylactic to preclude the development of infections which formerly claimed a very heavy toll. Coupled with this, and due to its low toxicity, there has been a constant tendency to increase the dosages employed in many disease conditions. Perhaps in large part resulting from this latter trend, penicillin-resistant bacteria have

not developed on an extensive scale, except in some hospitals where insensitive staphylococci and *Strep. viridans* are beginning to constitute a problem.

Penicillin remains the drug of choice for combating infections caused by pneumococci, including pneumonia, empyema, and meningitis; streptococci, including hemolytic and non-hemolytic types, scarlet fever, and meningitis (with sulfonamides); staphylococci, including bacteremias, and more localized infections; gonococci; anthrax bacillus; and the spirochetes of syphilis and yaws. In conjunction with specific antisera it is used in diphtheria, gas gangrene, and tetanus. Its use in peritonitis has been largely supplanted by the broad spectrum antibiotics.

Dosage forms have changed appreciably. In the early days penicillin was given almost entirely by continuous intravenous instillations or 3- to 4-hourly intramuscular injections, the drug being administered generally as an aqueous solution of the sodium salt. In 1944 Romansky and Rittman found that absorption of the drug was much less rapid when it was incorporated in a mixture of peanut oil and beeswax, and for a few years thereafter this formulation was much used for daily intramuscular injections. It was especially suitable as a 300,000 unit "one-shot" treatment for gonorrhea. More recently pharmaceutical laboratories have succeeded in combining penicillin and procaine to produce a procaine salt of penicillin which, because of low solubility, insures prolonged maintenance of suitable blood levels. This is commonly administered as an aqueous suspension or in oil with 2 percent aluminum monostearate. Procaine penicillin has become so generally used that 70 to 75 percent of the total output is currently produced in this form. The oral administration of penicillin as buffered tablets is steadily increasing, with adequate blood levels being obtained when 3 to 5 times the amount needed for parenteral administration is given. Many devices for inhalation therapy have been developed and an appreciable amount of penicillin is used in this way. Additional amounts are used in ointments and salves. There is a fairly large outlet in the form of veterinary preparations, and in recent months an increasing quantity has been used for feed supplementation with and without vitamin B<sub>12</sub>.

Penicillin is essentially non-toxic. Baron (1949) lists the LD50

in experimental animals as 1 to 2 gm./kg. of body weight. Toxic levels in man have seldom if ever been encountered. Keefer (1949) tells of having known one patient who received 100 million units daily for two weeks without side effects—this intake represented about 60 gm. of *pure* sodium penicillin per day. About 3 to 5 percent of the patients who receive penicillin become sensitized to it, such sensitivity commonly being manifest as skin eruptions. These reactions are most common when penicillin is administered in oil and wax. Sensitization may arise also from exposure to the drug and occurs not uncommonly among the personnel in manufacturing plants.

Penicillin presents a paradox. It was the first therapeutically effective antibiotic discovered; still, it remains the least toxic and the most useful, despite the fact that more than 300 additional ones have been discovered. It is produced by common saprophytic molds; yet, it remains the only useful antibiotic derived from truly filamentous or fleshy fungi whose toxicity will permit systemic use, although more than 130 antibiotics of such origin have been reported subsequently. When one speaks of penicillin as a miracle drug, one need not consider solely its curative properties; one may just as aptly refer to its discovery and its source. Equal to its importance as a drug has been the effect of precipitating and sustaining the unprecedented search for other drugs of microbial origin. Everywhere the searchers say: "If it can happen once, surely it can happen again."

#### STREPTOMYCIN

For more than three decades the Actinomycetes have received special study by Professor S. A. Waksman at Rutgers University. Beginning in the late '30's such investigations were intensified, and particular attention was given to problems relating to antagonism between microorganisms. The actinomycetes exhibit this phenomenon with unusual frequency, a fact already known to Waksman and to soil microbiologists generally. It was natural that his attention should become focused on them. I doubt, however, if anyone viewing the future objectively in 1941, and with penicillin and tyrothricin already to the fore, could have predicted that of the next four clinically outstanding antibiotics to be discovered, all

would come from this single group of microorganisms. Reference is made, of course, to streptomycin, Chloromycetin, aureomycin, and terramycin.

Before pursuing our story of the discovery and large-scale development of these antibiotics, let us, as mycologists, briefly consider the microbes from whence they come. It is an understatement of the first order to say that we have neglected them. Sensing this neglect, the bacteriologists have adopted them—halfheartedly. But is it their brood to which the actinos actually belong? They look like delicate molds—and Heaven knows they smell like molds! Whatever their relationship, permit me to focus your attention on a second paradox. No other group of microorganisms is today being searched for the presence of new and valuable products of metabolism with equal intensity, when at the same time no other group is, to my knowledge, so inadequately understood. Is it not symptomatic when the new antibiotics, with very few exceptions, always come from new species? It is a very big job, and it is not to be undertaken lightly, but an enviable and lasting reputation awaits the investigator who, with patience and skill in the application of accepted mycological techniques coupled with the introduction of adequate physiological tests, makes a comprehensive study of the order Actinomycetales. Never before has there been available for study a body of living cultures equal to the number of actinomycetes currently maintained in the research laboratories of the drug industry. Some highly significant studies are already under way. Applying techniques common to the study of the more delicate aquatic phycomycetes, Professor John Couch (1950) has succeeded in demonstrating that actinomycetes formerly diagnosed as *Micromonospora* are capable of developing enlarged zoosporangia from which escape, upon rupture, large numbers of minute swimming spores. Even though the spores appear bacterial in character, it is a most umbacterial type of behavior.

The first antibiotic of actinomycetous origin, actinomycin, was reported by Waksman and Woodruff in 1940. This was obtained from a new species, *Streptomyces antibioticus*, and *in vitro* was especially active against grani-positive cocci and bacilli. It proved to be very toxic to experimental animals, hence was never considered as a potentially useful drug. A second antibiotic, strepto-

thricin, was reported a year later from the same laboratory (Waksman *et al.*, 1941). This was derived from another species, *Streptomyces lavendulae*. It appeared at first to offer considerable promise in some infections, only to show a delayed toxicity which precluded clinical use. A third and most useful antibiotic, streptomycin, was first announced by Schatz, Bugie, and Waksman in January 1944. This was produced by strains of *Streptomyces griseus* (Krainsky) Waksman and Henrici, newly isolated from soil, and exhibited a very favorable bacterial spectrum, being active against gram-negative as well as gram-positive species. It showed substantial activity against *Mycobacterium tuberculosis*. Toxicity was comparatively low; the LD<sub>50</sub> in mice being about 0.75 gm./kg. of body weight when administered subcutaneously. Maximum blood levels were attained within 30 minutes. In animal experiments, streptomycin was found to give good protection against infections with gram-negative pathogens insensitive to penicillin. It thus appeared probable that in streptomycin the clinician would have an antibiotic to complement the action of penicillin.

Clinical studies conducted at the Mayo Clinic and by the National Research Council's Committee on Chemotherapeutics and Other Agents (Keefer, 1946) established the efficacy of streptomycin in the treatment of tularemia, in *Hemophilus influenzae* infections, and in urinary tract infections and bacteremias caused by gram-negative bacteria. Streptomycin was found to have a suppressive effect upon the growth of the tubercle bacillus in man.

As information of this type accumulated, many pharmaceutical firms already manufacturing penicillin took up the study of streptomycin and undertook its production. Capitalizing upon the experience gained in adapting the submerged culture process to the penicillin fermentation, manufacturers were able to achieve large-scale production of streptomycin almost as rapidly as fermenter capacity became available.

In their early papers Waksman and co-workers used a production medium based upon glucose, peptone, beef extract, and sodium chloride. For large-scale production a cheaper substrate was essential, and soy-bean meal has come to represent an important constituent, replacing peptone and beef extract as sources of nitrogen. In addition to dextrose, limited amounts of some growth supplement,

such as distillers solubles or corn steep liquor, and calcium carbonate are commonly used. Streptomycin is produced aseptically in large steel or stainless steel tanks with agitation and aeration in much the same manner as penicillin; and fermentation equipment can, in general, be interchanged between the two fermentations as the market situation dictates.

Freshly isolated strains of *Streptomyces griseus* usually produce streptomycin in varying amounts, whereas cultures long maintained as laboratory stocks commonly lose such capacity. This can, however, be reestablished by the appropriate use of mutagenic agents (Waksman and Harris, 1949). Whereas strain improvement in *S. griseus* has not been so dramatic as in the case of *P. chrysogenum* cited above, substantially increased productivity has been achieved by the application of X-ray and ultraviolet radiation and exposure to nitrogen mustard compounds. Dulaney *et al.* (1949) obtained mutants producing more than 3.2 times as much streptomycin as the parent culture by the use of ultraviolet, nitrogen mustard ( $\text{ClCH}_2\text{CH}_2)_3\text{N}$ , and ultraviolet in successive experiments. Savage (1949) likewise secured substantial improvement in streptomycin productivity, using X-ray and ultraviolet radiation as mutagenic agents. Actinophages have presented a serious problem in streptomycin production; this difficulty has been largely overcome by the development of phage-resistant strains.

Benedict *et al.* (1950) isolated from Japanese soil a new species of *Streptomyces*, *S. griseocarneus*, which produces an antibiotic differing from streptomycin by one hydroxyl group. The chemistry of this compound has been carefully investigated by Stodola *et al.* (1951), who have applied to it the name, hydroxystreptomycin. Concurrent with this work, investigators at the Abbott Laboratories discovered and characterized the same antibiotic (Grundy *et al.*, 1950). It was originally hoped that hydroxystreptomycin might retain the desirable properties of streptomycin but show less toxicity and exhibit a lessened tendency to promote the development of resistant strains, but pharmacological tests have failed to substantiate this optimism. Bacteria that are resistant to streptomycin are also resistant to hydroxystreptomycin, and *vice versa*.

Streptomycin production has risen steadily since January 1946 when the drug first became available, the current monthly output

ranging in the neighborhood of 12 million grams. Whereas the unit of streptomycin was early placed upon a weight basis, approximating one microgram, the tendency has been to steer away from the use of fantastically large figures, as is still the practice with penicillin, and to recommend dosages upon a grams-per-day basis. Much convenience is thus gained in the routine use of the drug. However, one cannot help but wonder if an important psychological fillip is not lost thereby. All of us have heard individuals say, with no little element of pride, that they have just received  $n$  million units of penicillin. I submit, may not the fact that one can take millions of units of anything represent *prima faciae* evidence that one possesses great inherent vitality?

Streptomycin is generally considered the drug of choice in the treatment of tularemia. It is especially valuable in *Hemophilus influenzae* and *Klebsiella pneumoniae* infections, and in diseases caused by the coli-aerogenes group. It is commonly used to combat a variety of sulfonamide- and penicillin-resistant infections. In combination with penicillin it finds application in the treatment of mixed infections of the urinary and upper respiratory tracts, and in cases of endocarditis failing to show a satisfactory prognosis with penicillin alone. In combination with polymyxin and bacitracin, two antibiotics of bacterial origin, streptomycin has been recommended as a preoperative prophylactic in intestinal surgery. Its greatest application, however, is in the treatment of tuberculosis in its various forms, and upwards of 70 percent of the available drug supply is used for that purpose. Although streptomycin is not an ideal drug, it is the most effective chemotherapeutic agent known for the treatment of tuberculous infections in man. It is especially important in alleviating the symptoms of many forms of the disease, allowing more time for other types of therapy. Formerly, comparatively large doses were administered, these often ranging from 2.0 to 4.0 gm./day, *via* intramuscular injections twice daily, and continuing from 2 to 4 months or longer. When used for extended periods, streptomycin shows considerable neurotoxicity. This is generally manifest as a disturbance of the eighth nerve, causing either vestibular dysfunction or, in some cases, deafness. The unfavorable response is intensified in patients suffering from renal insufficiency.

Attention was early directed toward dihydrostreptomycin (prepared from streptomycin by catalytic hydrogenation), and comparative clinical trials indicated substantially less toxicity for this derivative than for the chloride or sulfate salts. Since 1948 an increasing proportion of the streptomycin has been marketed in the dihydro form, which today accounts for about 80 percent of production. Treatment of tuberculous patients with a regimen of interrupted streptomycin (once or twice weekly) and daily administrations of para-aminosalicylic acid (PAS) has tended to reduce further the incidence of serious neurotoxic symptoms.

Use of streptomycin and para-aminosalicylic acid therapy has resulted in a second and perhaps even more important advance. It has reduced very substantially the development of tubercle bacilli that are resistant to streptomycin, for as in the case of neurotoxicity, drug resistance is directly related to daily dosage and duration of treatment. Streptomycin-resistant forms almost invariably emerge when substantial amounts of the drug are given daily for a matter of months, and such insensitive forms may grow in concentrations of streptomycin up to 10,000 times an amount that was initially inhibitory. Occasional strains are streptomycin-dependent, and continued treatment in such cases may actually favor the infection. Discussing the problem of streptomycin resistance in *Mycobacterium tuberculosis*, Dr. Esmond R. Long has recommended (1949) that intermittent relatively high doses of dihydrostreptomycin be employed (to capitalize upon prompt and maximal drug action) in connection with some other chemotherapeutic agent (PAS) capable of suppressing the growth of the tubercle bacillus. The statistical probability for the occurrence of a mutant tubercle bacillus resistant to both streptomycin and another inhibitory drug is very small indeed. A set of principles for chemotherapy in tuberculosis which represents the views of most clinicians is set forth in the May, 1951, issue of the *American Review of Tuberculosis*. It is in complete agreement with Long's recommendation.

The phenomenon of streptomycin resistance is by no means limited to *Mycobacterium tuberculosis*. It is, however, more acute in this than in most other infections because of the extended treatments that are required.

Streptomycin is usually administered by intramuscular injection. It is not absorbed from the intestines but may be used orally to combat infections of the alimentary tract. It is sometimes used in the form of an aqueous spray to combat infections of the respiratory tract. It is seldom, if ever, used in salves or ointments because of its great propensity for causing dermatitis.

Investigators in many laboratories are searching frantically for some microbial drug which will possess the curative properties of streptomycin, but lack its weaknesses. Two antibiotics have been discovered and suggested for this role. The first of these, neomycin, produced by *Streptomyces fradiae*, was announced by Waksman and Lechevalier in 1949. *In vitro* activity was good, even against streptomycin-resistant forms of the tubercle bacillus. However, limited clinical trials to date have not established its superiority over streptomycin; on the contrary, transient renal damage is observed and progressive deafness is not uncommon, whereas resistant forms emerge just as they do to streptomycin. The second contender, viomycin, apparently produced by a *Streptomyces* of unsettled identity, was discovered almost simultaneously by investigators at Parke, Davis and Company and the Pfizer Company. Again, *in vitro* inhibition of the tubercle bacillus is good, animal experiments are promising, but available clinical data fail to manifest a superior drug.

#### THE BROAD SPECTRUM ANTIBIOTICS

Penicillin is an invaluable drug for the treatment and cure of many serious bacterial infections, especially those caused by pyogenic cocci, pneumococci, gram-positive bacilli, gonococci, and the spirochetes. Streptomycin complements the action of penicillin, and, despite its serious limitations, constitutes the best chemotherapeutic agent available for the treatment of tuberculosis and certain infections caused by gram-negative bacteria. Important as are these two microbial drugs, they still leave unchallenged a multitude of dreaded infectious diseases. In the years since 1947, three additional antibiotic drugs have been discovered which further narrow (FIG. 1), but do not obliterate, the retreating host of uncontrolled infections. These so-called broad spectrum drugs, *Chlorampheni-*

*col*, *aureomycin*, and *terramycin*, have much in common. All are produced by species of *Streptomyces*, and all are characterized by their ability to inhibit both gram-negative and gram-positive bacteria, the rickettsia (intermediate between the bacteria and true viruses), certain large viruses and many bacteria that are resistant to penicillin and streptomycin—hence the designation, broad spec-

## OVERLAPPING IN VITRO ACTION OF ANTIBIOTICS

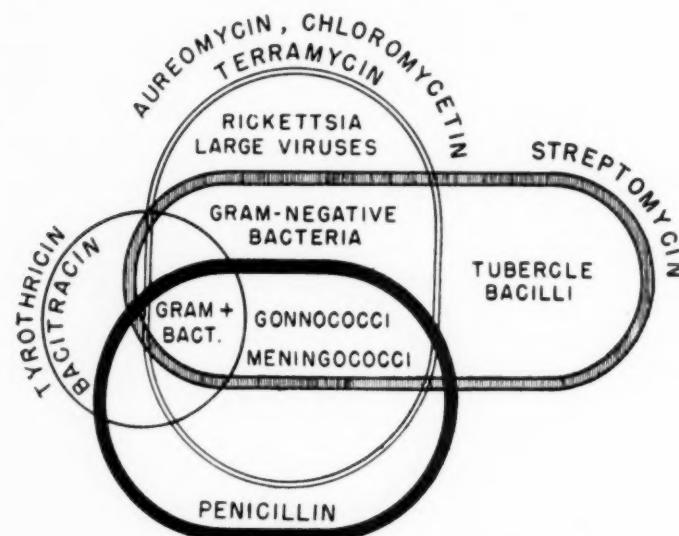


FIG. 1. Diagram showing the types of infections in which the different antibiotics are most effective. (After Pulaski, Ann. N. Y. Acad. Sci. **53**: 348. 1950.)

trum antibiotics. Each of these was discovered independently, and each seems to be finding some field of special application. For these reasons, I shall introduce them separately. However, their similarities outweigh their differences (Welch, 1950), and in the treatment of certain diseases there is little to choose among them. I should think that the physician's choice of a drug might well depend upon which clinical report, or brochure, he last read. From

the intensity of the promotional campaigns now being waged, I suspect that the manufacturing companies think likewise. As Americans, we can take comfort from the old adage, "Competition is the life of trade," and in this case it will undoubtedly lead to increased drug supplies at lower cost. Individually, we should be most happy that three broad spectrum antibiotics are available instead of one. As we shall subsequently see, competition combined with technical improvements in manufacturing processes has already reduced the cost of penicillin and streptomycin to a fraction of original levels.

#### CHLORAMPHENICOL

The discovery and preliminary chemical characterization of chloramphenicol, or Chloromycetin<sup>3</sup> as it is generally known, was announced by John Ehrlich and co-workers of Parke, Davis and Company and Paul R. Burkholder of Yale University in October 1947. The antibiotic was obtained from a species of *Streptomyces* isolated in Burkholder's laboratory from a sample of mulched soil collected near Caracas, Venezuela. Early fermentation and chemical studies were carried out in Detroit. Quite independent of this discovery, David Gottlieb and co-workers had isolated from a compost soil at the University of Illinois an unnamed species of *Streptomyces* which produced an antibiotic apparently identical with chloromycetin (1947). In the following year, the two research teams published jointly a paper entitled, "*Streptomyces venezuelae* n. sp., The Source of Chloromycetin." Investigators at Parke, Davis and Company (Smith *et al.*, 1948) have published a fairly full account of the biological aspects of chloromycetin production. From the begining, studies were conducted in submerged (shaken-flask) cultures, and early trials indicated the beneficial effect of glycerol as a source of carbon and selected meat products as a source of nitrogen. The fermentation was further improved by the addition of distillers solubles or molasses. Chloromycetin was found to inhibit a wide variety of bacterial species, including gram-negative and gram-positive forms. It was quite active against rickettsia in chick embryos, being the first antibiotic to offer promise in this field. It was relatively inactive against the mycobacteria and was inactive

<sup>3</sup> Registered trade-mark, Parke, Davis & Co.

against fungi. Oyaas *et al.* (1950) have reported the following as a favorable laboratory-scale medium: glycerol, 1 percent; tryptone, 0.5 percent; B-Y fermentation solubles, 0.5 percent; and sodium chloride, 0.5 percent. The pH is adjusted to 7.5 with NaOH prior to sterilization, and the fermentation proceeds at a slightly alkaline pH. Large-scale production by fermentation process is carried out aseptically in large tanks with aeration and agitation much like the penicillin and streptomycin fermentations.

Synthesis of Chloromycetin was reported in April, 1948, at the San Francisco meeting of the American Chemical Society by Crooks, Rebstock, and others; and primary emphasis has been placed on this mode of production. Currently, more than half the available chloromycetin is produced by chemical synthesis, and a new plant, now under construction, will employ this method exclusively. Chloromycetin is the first and only antibiotic drug for which a practical synthesis has been worked out. Clinically, Chloromycetin of synthetic and fermentative origins are equally effective.

Extensive clinical trials by Smadel and others (1948, *et seq.*), and subsequent wide clinical use of the antibiotic, have established it as a very useful therapeutic agent. It is the drug of choice for the treatment of typhoid fever, and it finds important application in other gram-negative infections of the gastro-intestinal and urinary tracts. In mixed surgical infections it extends the range of penicillin yet does not pose the hazard of streptomycin. It is especially useful in the treatment of rickettsial infections such as Q-fever, Rocky Mountain spotted fever, typhus, scrub typhus, and the like. It can be used in primary atypical (viral) pneumonia and produces a favorable response in whooping cough (*Hemophilus pertussis*). It is active against gonococci and spirochetes, but use in these diseases is limited to penicillin-insensitive infections.

Chloromycetin is relatively non-toxic. The drug is administered orally, and dosages generally range from 1.0 to 4.0 gm./day, depending upon the nature of the disease and the severity of the infection. Microorganisms showing resistance to Chloromycetin can be developed in the laboratory, but such have not as yet presented a problem in clinical practice.

## AUREOMYCIN

The discovery of aureomycin by B. M. Duggar and associates of the Lederle Laboratories was announced at a Conference on Ayreomycin held by the New York Academy of Sciences in July, 1948.<sup>4</sup> The antibiotic is produced by a new species of *Streptomyces*, *S. aureofaciens*, which was first isolated from a sample of soil collected from a timothy field in Missouri. At certain stages of growth the mold is characterized by a golden yellow mycelium and the isolated antibiotic is similarly colored, hence the species and product names. Aside from Duggar's patent, which is couched in the language and generalities "known to those experienced in the art," little has been reported regarding the conditions under which the fermentation is carried out. For "larger-scale production" (200 liters), a fermentation medium of the following composition is cited: Corn steep liquor, 1 percent; sucrose, 1 percent;  $(\text{NH}_4)_2\text{HPO}_4$ , 0.2 percent;  $\text{KH}_2\text{PO}_4$ , 0.2 percent;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.025 percent; and  $\text{CaCO}_3$ , 0.1 percent. The pH of the medium after sterilization is 6.0 to 6.1, and during fermentation it drops to about 4.5 to 4.8. The fermentation is conducted in large aerated tanks with agitation as in the manufacture of other antibiotic drugs. Antibiotic titers of fermented liquors are not disclosed. It is known, however, that substantial strain improvements have been realized in this fermentation by the repeated exposure of cultures to different mutagenic agents. We can assume that the fermentation now proceeds at a highly profitable, if not a wholly satisfactory, level. Some specific actinophages have been encountered, which are apparently controlled by the use of phage-resistant strains and adherence to strict asepsis throughout the fermentation.

Aureomycin is now produced in very large amounts, and is generally available to all physicians. A massive clinical literature has already been built up, and the consensus seems to have been well stated by Herrell, who, in reviewing the newer antibiotics (1950),<sup>5</sup> wrote as follows:

<sup>4</sup> Papers presented at this Conference were subsequently published in the Annals of the Academy, November 30, 1948.

<sup>5</sup> It should be noted that very little clinical data were yet available on terramycin when this article was prepared.

"Published studies to date dealing with aureomycin leave one with the inescapable impression that this antibiotic is probably the most important antibiotic developed since the introduction of penicillin."

The toxicity of aureomycin is very low, and relatively large doses can be given with few side effects except for occasional nausea and vomiting when sustained doses are administered.

The drug is supplied as the hydrochloride and administration is nearly always by the oral route. Dosages commonly range from 1.0 to 3.0 gm. daily, divided in 6-hourly doses. Aureomycin may be administered intravenously if the clinical picture demands, in which case the dose is reduced very substantially.

Aureomycin possesses great penetrating power, being absorbed into the central nervous system, bone joints, etc., more readily than most other microbial drugs. For this reason it may find application where the specific infection might otherwise indicate penicillin or some other antibiotic.

Aureomycin is considered by many to represent the drug of choice for the treatment of the various rickettsial diseases and infections caused by the psittacosis-lymphogranuloma-venereum group, primary atypical (viral) pneumonia, and for a variety of infections caused by penicillin-resistant cocci, micrococci, and spirochetes. It is effective in *Klebsiella pneumoniae*, *Haemophilus influenzae*, and *Pasteurella tularensis* infections. It is widely used to combat mixed infections of the gastrointestinal and urinary tracts. In combination with dihydrostreptomycin it affords a promising treatment for brucellosis. It exerts an ameliorative effect in whooping cough. It may be used advantageously in the treatment of peritonitis, in severe cases often being combined with penicillin. It has been used effectively in the treatment of actinomycosis. Some reports indicate its usefulness in viral infections such as infectious mononucleosis and influenza, but additional experience must determine whether the favorable response is due to a direct effect upon the etiological agent or to the exclusion of secondary manifestations. Aureomycin is effective in the treatment of amebic dysentery, possibly exerting its effect by killing off the bacteria upon which the amebae feed. For the same reason it provides a favorable pre-surgery treatment of the alimentary tract.

One of the greatest potential weaknesses of aureomycin and other

broad spectrum antibiotics may arise from this very ability to effectively "sterilize" the alimentary tract. Patients subjected to extended antibiotic treatment by the oral route may develop diarrhea with stools containing a heavy growth of *Candida albicans*, and, unless therapy is corrected accordingly, may end up with a "cure" in which the side effects are as disagreeable as the initial disease. Occasional deaths due to *Candida* infections (moniliasis) of this origin have been reported.

In addition to its outstanding performance as an antibiotic in the true sense of this word, aureomycin has assumed added importance since Jukes and Stokstad (1950) demonstrated it to effect profound growth stimulation in birds and other animals.

#### TERRAMYCIN

The third broad spectrum antibiotic, terramycin, represents the culmination of an intensive screening program conducted by the Charles Pfizer Company. It was first announced in a brief paper in *Science* by Finlay *et al.* in January, 1950. In the following June a Conference on Terramycin<sup>6</sup> was held by the New York Academy of Sciences, and extensive information regarding its biological properties, pharmacology, and clinical indications was revealed. I think it can be safely said that in no other instance has the discovery of an antibiotic resulted from a planned campaign more extensive in scope or singular in its ultimate objective.

Terramycin represents a metabolic product of a new species of *Streptomyces*, *S. rimosus*, obtained from soil of unstated origin. More than 100,000 samples of soil from all accessible parts of the world were examined, molds were isolated in pure culture, and these were surveyed in small shaken flasks for the possible production of interesting and significant antibiotic agents (Kane *et al.*, 1950). Little is known regarding conditions which favor the fermentation aside from the general information which is given in the terramycin patent (Sobin *et al.*, 1950). The following example is cited as a medium suitable for production: Soybean meal, 3 percent; corn starch, 0.5 percent; N-Z-Amine B, 0.1 percent; NaNO<sub>3</sub>, 0.3 percent; CaCO<sub>3</sub>, 0.5 percent; and vegetable oil (to control

<sup>6</sup> A report of the Conference was published in the Annals of the Academy, September 15, 1950.

foaming), 0.4 percent. The pH is adjusted to 7.0 prior to sterilization and rises to pH 8.0 as the antibiotic titer reaches its maximum. As in the microbiological production of other antibiotics, the fermentation is carried out aseptically with agitation in large aerated tanks. The isolated drug is apparently characterized by unusual chemical stability.

Terramycin is marked by low toxicity. Side effects are apparently limited to gastrointestinal disturbances. Unlike aureomycin it does not diffuse readily into the cerebrospinal area, hence behaves in a manner rather comparable to penicillin. It does, however, diffuse readily into the pleural cavity. The drug is marketed as the crystalline hydrochloride primarily, and administration is usually *via* the oral route. It may be given intravenously if desired, in which case it is used with sodium glycinate as a buffer. It is available also in ointments, troches, and as an elixir for pediatric use. The drug is currently produced in large amounts.

Terramycin became available for clinical use in the spring of 1950, and already a substantial literature has been built up covering a wide variety of infections. In general, its range of applicability approximates that of aureomycin, hence a detailed enumeration is not necessary. Especially favorable responses have been obtained in the treatment of amebiasis and brucellosis. Infections due to *Bacteroides* have responded satisfactorily and the drug has been used successfully in bronchiectasis. It is preferred by some clinicians for the control of mixed bacterial infections of the gastrointestinal and urinary tracts. Striking cures have been obtained in penicillin-resistant syphilis and in yaws. Terramycin is ineffective against *Proteus vulgaris* and *Pseudomonas aeruginosa* infections. Clinical trials in several hospitals to date have failed to substantiate the early hope that terramycin might prove an effective agent for the treatment of tuberculosis.

Resistance to terramycin can be developed in different bacterial species by growing the organisms in the presence of increasing concentrations of the drug. Interestingly enough, strains resistant to terramycin show a parallel resistance to aureomycin and may show some resistance to Chloromycetin (Fusillo and Romansky, 1951).

When incorporated in animal feeds, terramycin evokes a growth response comparable to aureomycin, penicillin, and bacitracin.

#### ANTIBIOTICS OF BACTERIAL ORIGIN

Many potent antibiotics of bacterial origin have been described. However, no one of these has yet achieved the stature of a major drug.

Of the various bacterial groups, the genus *Bacillus* is particularly productive, no less than 35 antibiotics having been identified from this single genus. Without exception, the antibiotics produced by species of *Bacillus* represent polypeptides, and when used parenterally exhibit nephrotoxicity. Antibiotics of this class commonly exhibit high antibacterial activity; and, if employed for purposes which preclude admission to the blood stream, may find considerable application. Of the various antibiotics of bacterial origin which find important if somewhat limited uses, the following may be considered: Tyrothricin, Bacitracin, Polymyxin, and Subtilin.

*Tyrothricin*, as already mentioned, was one of the first antibiotics to be isolated and characterized (see p. 5). It is produced by a soil bacillus, identified as *Bacillus brevis*. Early studies showed it to possess great antibacterial activity, especially against the gram-positive cocci, and its possible use in combating infections of this type was suggested by Dubos. Too great toxicity has precluded its use for the treatment of systemic infections; it has, however, found application as an antiseptic in medicated bandages and surgical dressings, and in preparations for the treatment of upper respiratory tract infections.

*Bacitracin*, first described by Johnson *et al.* (1945), was obtained from an unidentified *Bacillus* isolated from a purulent wound. More recently it has been identified by Nathan R. Smith as probably representing *B. licheniformis*. The antibiotic is a polypeptide which accumulates in the culture liquor free from the bacterial cells. It is non-irritating to living tissue, but its nephrotoxicity limits its use in systemic infections. It is approved by the Food and Drug Administration for general topical use and finds application in the treatment of superficial lesions, surgical infections, and for the relief of respiratory congestion. In May 1951, it was ap-

proved for parenteral use "in hospitals only," where patients can be constantly observed for signs of developing nephrotoxicity. It is a life-saving drug in some infections due to bacteria insensitive to other antibiotics. In combination with streptomycin and polymyxin B, it is used for "sterilizing" the gut prior to intestinal surgery. In combination with neomycin (under the trade name, Neobacin), it is recommended for use in idiopathic diarrhea. It is valuable also as an antibiotic feed supplement, comparing very favorably in this regard with aureomycin, penicillin, and terramycin. Bacitracin is now produced in fairly large amounts, and it remains on the borderline as a potentially important drug. Efforts are being made to modify it in some way to reduce its toxicity.

*Polymyxin*, discovered independently by Benedict and Langlykke (1947) at the Northern Regional Research Laboratory and by Stansly *et al.* (1947), American Cyanamid Company, is produced by various strains of *Bacillus polymyxa*. Almost simultaneously Ainsworth *et al.* (1947) of Wellcome Laboratories in England discovered the same antibiotic, applying to it the name, Aerosporin.<sup>7</sup> As in the case of the penicillins, "polymyxin" represents, in reality, a family of closely related compounds which differ quantitatively in their effect upon various sensitive bacteria. In general, gram-negative bacteria are strongly inhibited; and when first discovered, it was thought that polymyxin might contribute substantially to therapy in this field. Appreciable nephrotoxicity limits its use in systemic infections. Like bacitracin, it has been approved for "hospital use only." It is a life-saving drug in certain *pseudomonas* infections. As mentioned above, it has found some application in "combiotic" therapy. Polymyxin D has been shown to exert some growth stimulation in chicks.

*Subtilin*, an antibiotic produced by occasional strains of *Bacillus subtilis*, was discovered by Jansen and Hirschmann (1944) at the Western Regional Research Laboratory. Unlike bacitracin and polymyxin, this antibiotic for the most part remains in the bacterial cells, hence must be released by some appropriate treatment. It shows high antibacterial activity, particularly against gram-positive forms. Solubility in physiological saline and body fluids is very

<sup>7</sup> Registered trade-mark, Burroughs Wellcome & Co. Inc.

low. Like bacitracin and polymyxin, it is a polypeptide; unlike these, it is readily digested by trypsin and pepsin. This latter characteristic has suggested its possible use as a preservative for canned foods subjected to mild heating. Some promising results along this line have been obtained by workers at the Western Laboratory. However, gradual diminution in antibiotic activity, coupled with an inability to sterilize the product completely in the absence of conventional heat processing indicates that subtilin is not an adequate answer to the problem. Low solubility has precluded serious consideration of subtilin as a potential drug for human use.

#### FEED SUPPLEMENTS—A DOUBLE BONUS

A quarter of a century ago Minot and Murphy (1926) showed that pernicious anemia could be successfully treated with liver. In 1931 Strauss *et al.* prepared a liver fraction suitable for parenteral injection, and by 1935 such extracts were available to clinicians. By periodic administration of such extracts, pernicious anemia could be held in check, thereby preventing the usual neurological and gastrointestinal symptoms, in addition to correcting the unfavorable blood picture. Further progress in improving the extracts was slow because each batch had to be tested on a pernicious anemia patient in relapse. This was time-consuming, and the test subjects varied greatly.

A most important discovery was made by Shorb in 1947; she found that one of two factors essential for the growth of *Lactobacillus lactis* Dorner was present in refined liver extracts in almost linear relationships to their activity in pernicious anemia. Thus a potentially valuable and rapid assay for the so-called L.L.D. factor was available. In 1948, Rickes *et al.* of Merck and Company isolated minute amounts of a red crystalline compound from clinically active liver extracts; and Shorb was able to show that this material was highly effective in promoting the growth of *L. lactis* Dorner, assaying about 10,000,000 L.L.D. units/mg. Soon thereafter it was shown that this material showed positive hematological activity in patients with Addisonian pernicious anemia. The red crystalline compound was called vitamin B<sub>12</sub>, and its identity with the anti-anemia factor in liver was established. Delving into the chem-

istry of the substance, Rickes *et al.* (1948b) found that it contained cobalt, an element never before found in a compound of biological origin.

Parallel with this development, H. R. Bird (1948) and other animal nutritionists had found that a marked growth response in animals, particularly chicks, resulted from the feeding of liver extracts, fish meal, and cow manure. Further, some substance present in these crude materials was found essential to hatchability of hen's eggs. The name "Animal Protein Factor" (APF) became generally applied to the unknown substance. Ott, Rickes, *et al.* (1948) demonstrated that crystalline vitamin B<sub>12</sub> could replace the crude APF substances in promoting chick growth.

Rickes *et al.* (1948c) then made a far-reaching discovery—they found vitamin B<sub>12</sub> activity in the culture broths of various bacteria and actinomycetes, including *Streptomyces griseus*, the streptomycin-producing organism. Vitamin B<sub>12</sub> in crystalline form was isolated from *S. griseus* fermentations and found to have the same physical and chemical properties as crystalline B<sub>12</sub> from liver. Furthermore, vitamin B<sub>12</sub> from *S. griseus* was found to have the same anti-anemia and growth-promoting activity as the vitamin derived from liver. It was soon shown that vitamin B<sub>12</sub> was produced in other microbial fermentations. Culture filtrates from streptomycin and aureomycin fermentations, formerly discarded, were now concentrated and became the chief sources of crystalline vitamin B<sub>12</sub> for clinical use, and, in the form of APF supplements, for a rapidly expanding market in animal nutrition. Thus was realized the first bonus—a very profitable outlet for fermentation "wastes," which in some factories had previously presented a costly and bothersome disposal problem.

The increasing demand for vitamin B<sub>12</sub>-rich feed supplements could not be met by the antibiotics manufacturers. In many laboratories an intensive search has been made for other microorganisms capable of producing high yields of the vitamin, and a number of so-called "primary vitamin B<sub>12</sub> fermentations" have been proposed. One of these, based on the use of a selected strain of *Bacillus megatherium*, was developed at the Western Regional Laboratory by Lewis and co-workers. Another has been developed at the Northern Regional Laboratory by Harlow Hall and his

associates. This latter process is based upon the use of a strain of *Streptomyces olivaceus* isolated from Japanese soil, and vitamin yields up to 3  $\mu$ g./ml. are obtained in a suitable substrate based upon distillers solubles or thin stillage, byproducts of the distilling industry. Interestingly enough, the addition of 1.5 p.p.m. cobalt chloride is required to obtain maximum yields. At least two manufacturers are using this process to produce vitamin  $B_{12}$ -antibiotic supplements, formerly knowns as APF supplements.

The second bonus emerged in an equally interesting way. Feeding aureomycin fermentation APF supplements, Stokstad, Jukes, *et al.* (1949) of the Lederle Laboratories obtained a growth response in chicks over and above that attributable to their vitamin  $B_{12}$  content alone. Knowing that these supplements contained traces of aureomycin, they reasoned correctly that the antibiotic itself might be exerting some stimulating effect. A few months later they were able to show that this was indeed true (1950). When chicks were fed a diet containing adequate levels of vitamin  $B_{12}$ , an additional and substantial growth response was obtained by adding small amounts of aureomycin to the ration. In the presence of antibiotic the amount of required  $B_{12}$  is oftentimes reduced, thus the former is said to exert a "sparing action"; it does not, however, obviate the need for the vitamin. The feeding of vitamin  $B_{12}$  and antibiotic at optimal levels does not in any way alter the need for other vitamins of the B-complex.

Comparable growth-stimulating effects of vitamin  $B_{12}$  plus aureomycin were soon demonstrated for turkey poult by McGinnis *et al.* (1949), for young pigs by Jukes *et al.* (1949), and for weanling rats by Stern and McGinnis (1950). It is claimed that feed supplementation with an antibiotic-vitamin  $B_{12}$  concentrate benefits young calves, whereas the same may prove actually deleterious if fed to older ruminants. In such animals the native microflora of the rumen provides adequate vitamin  $B_{12}$ , and the presence of an antibiotic may modify this flora sufficiently to interfere with the animals' normal digestive processes. Egg hatchability is dependent upon vitamin  $B_{12}$  and is not enhanced by the addition of antibiotic alone.

Feeding tests have disclosed that other antibiotics will produce a growth response equal to aureomycin, and it appears that aureo-

mycin, penicillin, terramycin, and bacitracin are about equally effective. Streptomycin may be used, but a relatively greater concentration of this antibiotic is required. When penicillin is used in feed, it must be added as the relatively insoluble procaine compound, otherwise much of its potential effect is lost.

In all animals, the effect of vitamin  $B_{12}$ -antibiotic feeding is most pronounced in young individuals. Weight gains of 20 to 30 percent at 4 to 8 weeks over controls fed a vitamin  $B_{12}$ -antibiotic-deficient diet are not unusual in chicks and turkey poult. Birds on the supplemented diet are less subject to enteric diseases. An important economy in feed is realized. Birds reach marketable size 2 or 3 weeks earlier on supplemented diets, and maturity, as measured by egg production, may be hastened. In pigs the effects of  $B_{12}$ -antibiotic supplemented rations are much the same: dramatically faster growth, less disease, and earlier marketability without detectable loss in meat quality.

The amounts of vitamin  $B_{12}$  and antibiotic required to produce a maximum growth response vary with the type of diet and the prior dietary history of the animals under test. Norris *et al.* recommends 1.5 to 2.0  $\mu$ g. vitamin  $B_{12}$  per pound of feed for poultry on an all-mash diet, or double that amount if fed on grain. Jukes (1950) stated that the practical level of aureomycin for feeding was about 10 gm./ton of feed, and as supporting evidence cited experiments with chicks, turkeys and pigs. Penicillin (procaine), bacitracin, or terramycin are used at about the same level.

Much theorizing has been done as to the underlying causes of observed effects. It would appear that  $B_{12}$  acts in much the same way as other vitamins of the B-complex; that is, it effects direct stimulation of the animal that receives it. This is indicated by experiments where eggs characterized by poor hatchability showed good hatchability following injection of appropriate amounts of vitamin  $B_{12}$ . It is indicated also by experiments where hens on an all-vegetable diet produced eggs of good hatchability when they received vitamin  $B_{12}$  *via* intramuscular injection. The picture is not equally clear with regard to the antibiotics, for growth response is not observed *unless* the antibiotic is given orally. It is commonly assumed that the antibiotic alters the intestinal flora in some way to produce the beneficial effect. The following explanations have been suggested: (1)

Certain intestinal bacteria—e.g., enterotoxin-producing clostridia, are held in check—and supporting evidence has been published. (2) The total number of intestinal microorganisms is reduced, thereby lowering the competition between these and the host animal for nutrients—and evidence in support of the first part of this thesis is readily obtainable. (3) The antibiotic stimulates increased intestinal synthesis of vitamins by the microflora normally present—and supporting evidence has been offered. In sub-optimal flocks or herds subject to enteric infections, there can be little doubt that a partial effect of the antibiotic is to correct or mollify the diseases. That this, or any of the above, cannot represent the whole effect would seem indicated by a beautiful experiment performed by Stokstad and Jukes (1950). They prepared an alkali extract of aureomycin supplement which showed *no* microbiological activity when assayed against *Staphylococcus aureus*, yet they obtained a growth response when this was fed to chicks. Now this does not necessarily prove that *all* antibacterial activity against *all* the microorganisms of the gut had been destroyed. The experiment should be repeated starting with the crystalline antibiotic. It has undoubtedly occurred to many people, as it has to your speaker, that a truly definitive experiment might be conducted on germ-free birds in Dr. Reynier's laboratory at Notre Dame University—perhaps it is already under way. The interaction between antibiotic, the recipient animal, and its intestinal flora is undoubtedly complex. In proportion to its complexity, however, the resolution of this relationship should add to our knowledge of animal nutrition, and may conceivably contribute much to an understanding of drug action in fields far removed from the immediate problem.

#### AN AREA OF INTENSE STUDY

The biblical quotation, "Many are called, but few are chosen," aptly summarizes the search for antibiotic drugs during the past decade. Ten years ago not more than half a dozen antibiotic substances had been reported; today the number exceeds 300. Of these, only five have attained the stature of major drugs, namely: penicillin, streptomycin, chloramphenicol (Chloromycetin), aureomycin, and terramycin. A few others find limited applications and today remain on the threshold as potentially important drugs,

namely: tyrothricin, polymyxin, bacitracin, subtilin, neomycin, thiolutin, and viomycin. All the others, for one reason or another, are adjudged unpromising for therapeutic use. In many cases the level of activity is very low; in some, the substances are inactive *in vivo*; in most they are too toxic for use in animals and man. A few, too toxic for parenteral use, might be used for topical application but offer no advantages over other antibiotics equally effective but less dangerous. Five out of 300; the percentage is very low. But the occasional "strike" more than compensates for all the disappointments.

Taking a broader view, we have learned much from the decade's search. Never before have microorganisms of all types been subjected to such intensive physiological study. We have today a much better appreciation of the biosynthetic capacities of microorganisms and higher plants than could have been gained otherwise. Many new and interesting compounds have been identified. I am neither capable, nor is this the place to discuss this field, but I might call your attention to Professor Raistrick's Bakerian Lecture (1949) on mold biosynthesis as an example of the type of integrated information that is emerging. Viewing the field as a mycologist, it is increasingly evident that species identity and product identity are by no means synonymous. It is further evident that in a given species biosynthetic potential is by no means constant in all isolates or strains. The use of such criteria, therefore, becomes of very questionable significance in establishing taxonomic relationships, except in a very general way.

The production of antibiotic substances appears to be quite commonplace throughout most of the plant kingdom, these having been reported from various seed plants, lichens, many groups of fungi, the actinomycetes, and bacteria. Baron (1950) listed 141 different antibiotics, not including synonyms. There is, however, no place where all reported antibiotics are enumerated and it is impossible to set a definite figure. Nevertheless, with the help of my associates, Dorothy Fennell and R. G. Benedict, I have attempted to secure some idea of the number actually reported, and also the number that are actually different.

Searching all the sources available to us, we have been able to list

388 reported antibiotics, of which 33 are known to represent synonyms and 57 remain unnamed. Some of the latter, if fully characterized, will undoubtedly be found to represent antibiotics already known. For these reasons we have taken the number 300 as probably representing a fair estimate of the number of described antibiotics that are actually different. Considering the different plant groups, the greatest reported number, 116, have come from the filamentous saprophytic molds,<sup>8</sup> with the genera *Aspergillus* and *Penicillium* accounting for 75 of these. Among this latter number also are 25 of the 33 known synonyms, indicating that the antibiotics of this group have probably been studied more critically than most others. Of 86 reported antibiotics from bacteria, 35 are produced by species of *Bacillus* and 13 by the single species *B. subtilis*. There are 28 unnamed antibiotics—and an undetermined number of synonyms—of bacterial origin. Seventy-three reported antibiotics are recorded for the actinomycetes, of which 5 are unnamed and 4 represent known synonyms. The seed plants account for 44; the higher fungi, primarily basidiomycetes, 31; while the remainder are derived from lichens, yeasts, algae, and miscellaneous sources. All too often one encounters situations which can be summarized in this way: "An unnamed and uncharacterized antibiotic obtained from an unidentified *Bacillus*." The day is long past when science might benefit by the reporting of unnamed, and more particularly inadequately characterized, antibiotics from unspecified sources. The probability of "discovering" an antibiotic already studied by someone else increases daily. What can be done about it? The establishment of a national, or better still an international registry of antibiotic substances and the organisms which produce them could go far toward meeting this problem. With the development of paper chromatographic techniques much valuable comparative data can be obtained with minute samples. The real difficulty comes from the fact that such samples are generally not available, even from the investigator who reported the antibiotic. Patience and caution, not haste and priority, should become the watchwords in this area of work and publication.

\* P. W. Brian has recently published in *The Botanical Review* (June, 1951) an excellent critique on the production of antibiotics by fungi.

We cannot say how many investigators have worked with antibiotics during the past decade. Nor can we know in how many laboratories and hospitals research with antibiotics has been carried on. However, any worker in the field—microbiologist, chemist, or clinician—can tell you that a tremendous scientific literature has been built up. With not too definite criteria to guide us, we estimate that more than 18,000 research papers dealing with some aspect of antibiotic discovery, production, or use have appeared since 1940. A bibliography on Chloromycetin compiled by Parke, Davis and Company in April of this year numbered more than 750 titles. Aureomycin was announced in July 1948 and already the Aureomycin Bibliography compiled by Lederle Laboratories numbers more than 3000 titles. The numbers of published papers on streptomycin and penicillin, particularly the latter, are very much greater. Merck's supplemental bibliography on penicillin (January 1946) listed nearly 1000 papers for the period from August 1, 1944 to July 1, 1945. For such relatively unimportant antibiotics as gliotoxin and claviformin Raper and Thom listed 18 and 22 titles, respectively, in 1949. Few other fields of research have been marked by comparable activity.

Attention is still directed primarily toward seeking, producing, and evaluating antibiotics that are effective against *bacterial infections* in man. However, investigations in other fields already point to discoveries of wider significance. In many different laboratories mold filtrates are being tested in animals (usually chick embryos) for antiviral activity. At the Rockefeller Institute for Medical Research, and more recently at Merck and Company, R. E. Shope (1948) has demonstrated the production of a potent antiviral agent by a common species of *Penicillium*. Results are still inconsistent, but if the uncertainties which underlie its production can be resolved, a goal of tremendous consequence will have been reached. Taking a different approach, Robbins and Asheshov at the New York Botanical Garden are moving toward the same objective.

Culture filtrates and plant extracts are being screened continually for antifungal activity. Many substances have been found to possess this property, but none yet discovered combine high *in vivo* activity and low toxicity which permit their use

for systemic fungus infections in man. Such an antibiotic is urgently needed. The control of plant diseases by agents of this type represents a tremendous field, and already several investigations point to this possibility, with antibiotics such as actidione and clavacin showing promise for special applications. The possible development of new plant varieties characterized by naturally occurring substances inhibitory to pathogenic fungi is suggested by the work of Irving and Fontaine (1945).

A most important aspect of the work on antibiotics has been the emergence of teamwork on a scale never before realized, at least not in the biological sciences. Disciplines as far removed as mycology and physical chemistry, and clinical medicine and chemical engineering have joined hands to attain a desired goal. Development of the drug terramycin illustrates what can be achieved. We do not know when the first terramycin-producing strain of *Streptomyces rimosus* was first isolated and shown to have interesting potentialities. We do know that a patent application was first filed in October 1949; the first public announcement of the antibiotic appeared in *Science* in January, 1950; and a two-day conference concerned primarily with pharmacology and preliminary clinical trials was held in June of the same year. By March 1951 more than 250 papers had appeared wherein clinical trials were reported and the drug was generally available for any physician who wished to use it. How much sooner might we have had the drug penicillin if Fleming in 1928 could have called upon a like reservoir of informative and facilities. The example of terramycin is outstanding, but it is not unique. Aureomycin and Chloromycetin, also, moved rapidly from laboratory hopes to valuable drugs.

#### FACT, FIGURES, AND ESTIMATES

During the past decade a new and rapidly expanding industry has developed for the manufacture of antibiotic drugs. Prior to that time one was apt to think of industrial fermentation and the alcohol industry, in its various forms, as virtually synonymous. If one were a bacteriologist, he thought also of the acetone-butanol and vinegar fermentations. If he were a mycologist, he might, or might not, think of the various organic acid fermentations. Today,

irrespective of his specialty in microbiology, he must perceive think of the antibiotics industry, for the value of its annual production is rapidly approaching that of the alcohol industry.

During the first half of 1951 the monthly production of penicillin ranged between 23 and 33 trillion units, for a semi-annual total of more than 167 trillion units.<sup>9</sup> If we accept 40 cents per million units<sup>10</sup> as a reasonable wholesale value for bulk sales, the net worth of the product at this level of distribution figures out about \$67,000,000 for the six-month period. If we calculate its wholesale value as the packaged drug, this figure would, of course, be substantially greater. During the same period, monthly production of streptomycin and dihydrostreptomycin ranged between 10 and 12 million grams, for a half-yearly total of more than 68 million grams.<sup>9</sup> If we take 38 cents per gram vial<sup>10</sup> as a wholesale value, the net worth of streptomycins for the half-year turns out to be almost \$26,000,000.

No production figures are available on the newer broad spectrum antibiotics, since they are manufactured only by or for individual firms. However, a report compiled by the Chemical Division of the U. S. Tariff Commission (dated July, 1951) lists \$90 million as the value of antibiotics other than penicillin and streptomycin in 1950. The broad spectrum antibiotics represent all but a small fraction of this amount.

The dollar value of antibiotics exported from the United States is very substantial. For the years 1949 and 1950 figures are broken down as follows:<sup>11</sup>

Antibiotics, derivatives, and preps.	1948	1949	1950
Penicillin	—	\$39,742,000	\$46,392,000
Streptomycin	—	40,026,000	26,153,000
Antibiotics, other	—	6,054,000	20,724,000
Total	\$71,446,000	\$85,822,000	\$93,269,000

Antibiotics are finding an increasingly important outlet in animal feeds. It is estimated, again without substantiation, that the annual value of antibiotic-vitamin B<sub>12</sub> feed supplements may range from

<sup>9</sup> Certification by Food and Drug Administration, based on monthly reports.

<sup>10</sup> Price quotations from *Oil, Paint and Drug Reports*, Jan. to July, 1951.

<sup>11</sup> F.D.C. Reports, April 21, 1951.

\$20,000,000 to \$25,000,000. Their value to the farmer and poultryman would be far greater.

Starting from scratch in 1942, the valuation of buildings and equipment used for penicillin manufacture had reached \$25,000,000 by 1945 (Coghill and Koch). Since that time facilities have been increased all along the line, and four new major antibiotic fermentations have come into being. Figures on capitalization of current facilities for the manufacture of antibiotics are not available. However, a number of key individuals in the industry have been good

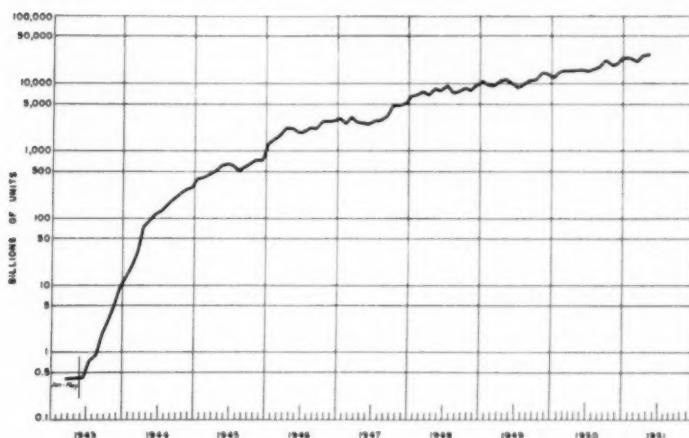


FIG. 2. Monthly production of penicillin in the United States from May 1943 through June 1951. (Data for May 1943 through October 1949 from Chemicals and Drugs, Industry Report, U. S. Dept. Commerce, Jan. 1950, p. 29. Data for Nov. 1949 through June 1951 from reports of monthly certifications of antibiotic drugs, Food and Drug Administration.)

enough to provide estimates on this point. A synthesis of their estimates seems to indicate a capitalization of approximately \$200,000,000 at the close of 1950. The industry is still expanding rapidly; the end is not in sight.

Along with the mounting production of penicillin and streptomycin has come a very dramatic decrease in price. These relationships are presented graphically in Figs. 2, 3, 4. When first sold to the Government in 1943 penicillin was priced below cost at \$20 per 100,000 units. By 1945 it was \$0.60 (Coghill and

Koch). Then started a slow decline to about \$0.04 in 1949, at which level it has since remained. The decline in streptomycin prices from \$30 per gram in 1945 to about \$0.35 to \$0.40 at present follows the same general course of an initial precipitous drop, a slow decline, and finally a leveling off. Even now, and despite so-called mass production, penicillin is an expensive compound, being valued at nearly \$300 per pound in bulk. When one considers that this amount of penicillin would fill more than 6000 vials with the conventional 100,000 units each, it becomes immediately apparent that initial cost no longer determines the price of the packaged drug. Cheaper penicillin, if available, would still be attractive to the feed industry. In the early days intense interest was shown in the possible chemical synthesis of penicillin, and such a synthesis was in time worked out by both American and British chemists. But the method was laborious and the product yield was very small; hence chemical synthesis was no longer considered as a competitor for the fermentation product.

The antibiotics represent an increasingly important part of prescription drug business. For the year 1950, Winn estimated that 25 percent of all prescriptions filled would represent drugs of this class. Further attesting to the magnitude of such retail sales, *The West Coast Druggist* for December 1950 lists the approximate

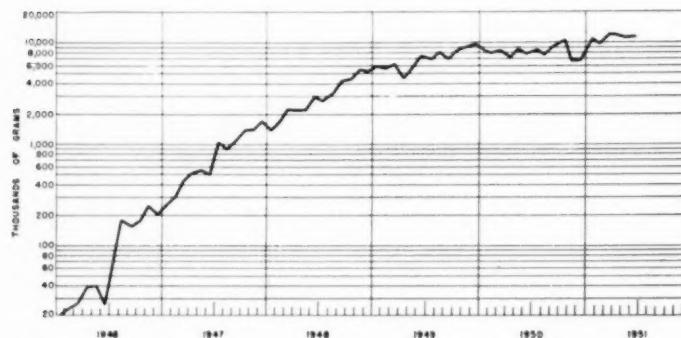


FIG. 3. Monthly production of streptomycin in the United States from January 1946 through June 1951. (Data for Jan. 1946 through Nov. 1949 from Chemicals and Drugs, Industry Report, U. S. Dept. Commerce, Feb. 1950, p. 34. Data from Dec. 1949 through June 1951 from reports of monthly certifications of antibiotic drugs, Food and Drug Administration.)

annual prescription sales of leading drug classes as follows: hormones, \$100 million; sulphonamides, \$150 million; vitamins, \$200 million; and antibiotics, \$250 million. Commenting about the increasing importance of antibiotics in the drug trade, McKeen (1950) stated:

"Some idea of the relative importance of this industry (antibiotics) may be gained by the fact that by 1948 the New York City Department of Hospitals was already expending over one-half of its total drug budget in the purchase of penicillin and streptomycin."

Any way you look at them, today antibiotics are big business.

By title, this discussion should be limited to the production of antibiotics in the United States, or at least in the Americas. However, I hope you will pardon me if I digress long enough to discuss briefly the interrelation of developments here and abroad, par-

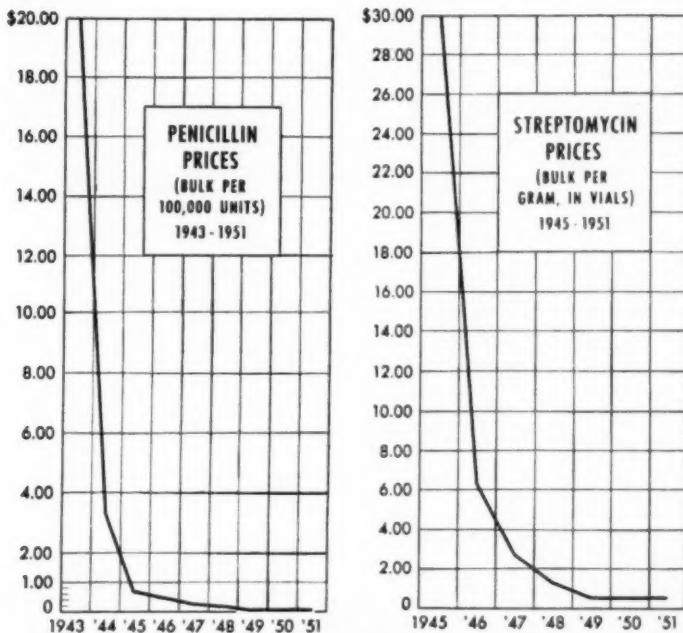


FIG. 4. Graphs showing declines in prices of penicillin and streptomycin from dates of introduction to March 1951. (From *Chem. and Eng. News* 29: 1192. 1951.)

ticularly in Great Britain. In 1950, McKeen estimated that eighty percent of the world's penicillin-producing capacity was located in the United States, the greater portion of the remainder being in the United Kingdom. Furthermore, to the best of my knowledge all foreign producers of penicillin use *Penicillium chrysogenum* Wis. Q-176, or strains derived therefrom, and all who can obtain them cheaply enough use lactose and corn steep liquor in their production media. Similarly streptomycin producers outside the United States employ cultures and processes (often licensed) developed here. The same is becoming true for Chloromycetin and aureomycin. Thus it can be said that the world's antibiotic industry, like our own, is based upon the developments which are traced in earlier parts of this report. Of this we are justly proud. But have we stopped to think what the course of events might have been had Fleming obtained a patent on penicillin? Undoubtedly he could have done so. And what if Florey and Heatley had filed a patent application before they opened conversations with investigators and manufacturers in this country? I shall not pursue the matter further—but I think all of us in America should mix some humility with our pride and realize that we have been privileged to contribute to a most important field under more than favorable circumstances.

#### ANTIBIOTICS AND NATIONAL HEALTH

Whereas antibiotics are important in animal nutrition, as catalysts of microbiological research and as items of commerce, their chief value stems, of course, from improvements made in national health. Each of us know individuals whose lives have been saved or extended by penicillin and other antibiotic drugs. The cumulative record of such cases projected on a national scale constitutes an impressive and generally favorable picture. In attempting to assess the value of antibiotics in improved health, it is perhaps best to consider separately the major diseases caused by different groups of microorganisms.

Infections caused by pneumococci were among the first to show a favorable response to penicillin. In recent correspondence, Dr. Chester Keefer<sup>12</sup> summarizes the present situation as follows:

<sup>12</sup> As Chairman of the Committee on Chemotherapy of the National Re-

"The overall fatality rate for *Pneumococcal pneumonia* in the pre-sulfonamide days without treatment with specific serum was 25 to 30 percent. When the sulfonamides were introduced, that is sulfapyridine, the fatality rate was reduced to 10 percent. Now with penicillin, it is less than 5 percent. The fatality rate in all pneumococcal infections has been reduced. Another example is pneumococcal meningitis which was 99 percent fatal before penicillin, now shows recovery rates of 50 percent. Pneumococcal endocarditis, a complication of pneumococcal pneumonia, had a fatality rate of 100 percent before penicillin and now shows a recovery rate of 25 percent."

Aside from actual death rates, Winn (1950) has pointed out that in the 1935-36 season the average hospitalization for pneumonia patients was over 19 days; today pneumonia commonly lasts no longer than a week and far fewer individuals require hospital care. Today, also, broad spectrum antibiotics are available for the few pneumococcal infections which do not respond to penicillin, as they are for the atypical pneumonias of viral origin.

Turning to the various diseases caused by streptococci, Keefer writes:

"With respect to streptococcal infections, it can be said that three striking things have been accomplished. (1) The fatality rate has been reduced in bacteremic cases from 85 percent to less than 10 percent. (2) Penicillin has prevented many of the complications of streptococcal infection so that when mild and moderately severe infections are treated, such complications as otitis media and mastoiditis do not appear. (3) The total duration of diseases like scarlet fever has been greatly reduced, carriers are eliminated, and the spread of the disease is controlled and complications prevented."

Discussing sub-acute bacterial endocarditis caused by non-hemolytic streptococci, Dowling (1948) states that cures can be obtained about 70 percent of the time with adequate and sustained penicillin therapy. The disease was almost invariably fatal prior to the introduction of this drug. The broad spectrum antibiotics are available for treatment of mixed bacterial and penicillin insensitive streptococcal infections.

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search Council Dr. Keefer directed the allotment and clinical evaluation of penicillin and subsequently of streptomycin.

Staphylococcal infections are now well controlled. Keefer has this to say:

"In staphylococcal infections fatality rates have been reduced in bacteremic cases from 85 percent to less than 10 percent, depending upon the location of the primary infection. Many complications are prevented. Many chronic cases of osteomyelitis are likewise prevented."

Dowling (1948) reports that fatalities due to staphylococcal osteomyelitis have been reduced to 1 to 2 percent. During World War II penicillin proved most useful in infections of this type. In staphylococcal meningitis the death rate is still very high (65 percent), but much improved. In endocarditis, invariably fatal in pre-penicillin days, recoveries may be expected in about half the cases.

Staphylococci become resistant to penicillin much more readily than most bacteria. Adequate dosages and prolonged treatment to avoid relapse are thus indicated. The seriousness of the problem is pointed out by Spink (1951). He reports that *in vitro* sensitivity tests to broad spectrum antibiotics can be used to indicate appropriate therapy in these cases.

The gonococci are among the most sensitive of all bacteria to penicillin, and even severe cases of arthritis and endocarditis of such origin have been treated successfully with this drug. For several years it has been known that gonorrhea can be cured in the great majority of cases by a single 300,000- to 500,000-unit dose of penicillin (see p. 14). The organisms are likewise sensitive to streptomycin and the broad spectrum drugs, so that the limited number of penicillin insensitive cases can be managed successfully with one of these. Still, the reported incidence, if not the number of infected individuals, is today much greater than it was in 1941 (TABLE I). Undoubtedly, a substantial portion of the reported cases represent reinfections. A probable explanation is found in the fact that a recognized cure is too prompt and too available.

In typhoid fever, the severity of the disease and the number of fatalities resulting therefrom have been reduced substantially by the advent and use of Chloromycetin and the broad spectrum drugs. Chloromycetin is especially beneficial in acute manifestations of

*Salmonella typhosa* infections; its efficacy in combating the carrier state is less clearly established. Dysentery and other enteric diseases caused by gram-negative bacteria are responding in a favorable but less spectacular manner to the broad spectrum drugs.

Rickettsial diseases such as Rocky Mountain spotted fever, Q-fever, typhus, and scrub typhus show a rapid and very favorable

TABLE I  
SYPHILIS AND GONORRHEA IN CONTINENTAL UNITED STATES, 1930-1950  
(Data from U. S. Public Health Service Reports)

Fiscal year	Syphilis		Gonorrhea	
	Cases reported	Deaths reported	Cases reported	Deaths reported
1930	213,309		155,875	
1931	229,310		155,729	
1932	260,564		158,083	
1933	234,647		149,527	
1934	230,890		153,255	
1935	254,551		161,810	
1936	266,626		162,487	
1937	336,147		182,435	
1938	480,140		198,439	
1939	478,738		182,314	
1940	472,900	19,006	175,841	419
1941	477,841	17,728	191,306	364
1942	472,245	16,345	212,384	298
1943	564,918	16,263	275,648	291
1944	458,199	14,916	300,585	241
1945	356,315	14,062	284,994	212
1946	360,918	12,995	364,853	150
1947	373,296	12,671	400,659	108
1948	338,141	11,616	363,014	71
1949	288,769		331,695	
1950	231,567		304,066	

1941-1950 Military cases excluded.

1930-1940 Military cases included.

response to all three of the broad spectrum drugs. The same drugs lessen the severity of various virus diseases, including atypical pneumonia. Some question remains as to whether observed effects result primarily from direct action of the drugs upon the infective viruses, or by preventing complications due to invading bacteria (Smadel, 1950).

In recent correspondence, Dr. Joseph E. Smadel summarized the current role of the broad spectrum antibiotics as follows:

"I think one can safely say that the mortality rate in typhoid and in the rickettsial diseases now approaches zero in those patients who are treated with the proper antibiotic before they are in extremis. . . . The period of convalescence from these diseases is now greatly shortened in treated patients. Thus, the febrile period lasts from 1-5 days after therapy is instituted, the duration depending upon the infection and the antibiotic. Hence, the disease is cut short in its progress and convalescence ensues earlier."

Amebic dysentery is effectually treated with either aureomycin or terramycin, but quantitative evaluation must await further use.

The picture with regard to streptomycin therapy in tuberculosis is apparently still somewhat clouded. There can be no doubt that the severity of the disease is often lessened, and further that this alleviation of acute symptoms affords valuable time for bed rest and other corrective measures. Recently I addressed the following question to Chester Keefer and to Esmond R. Long, both outstanding authorities on the treatment of tuberculosis. "What effect has the use of streptomycin had upon the incidence of and mortality from tuberculosis in the United States?" Their replies, in part, are given herewith.

Dr. Keefer reports:

"The fatality rates for tuberculosis for different years are as follows:

1940 . . . . .	43.7 per 100,000
1945 . . . . .	34.4 per 100,000
1950 . . . . .	21.0 per 100,000

It is difficult to say even now how much streptomycin has contributed to the reduction of the fatality rate in tuberculosis. However, there is no doubt that lives have been prolonged. Symptoms have been relieved and many patients have had an arrest of their disease who would have had a much longer illness."

Dr. Long has this to say:

"It is very difficult to determine if the use of streptomycin has had any effect as yet upon the death rate. The compound has been employed in the clinical treatment of tuberculosis for six years. During the first two years of that period relatively few cases were treated, and the results of treatment during the last year are not yet available in the literature. Therefore, we have data from only about three years on the basis of which to make a statement.

"The principal difficulty in determining the effect of streptomycin on mortality rates is the fact that the mortality rate from tuberculosis was dropping rapidly at the time streptomycin was introduced. ". . . many early cases of tuberculosis, detected in the acute stage, are definitely arrested with the help of streptomycin (you will note that I do not say *by* streptomycin); and in many others, of a more advanced nature, the disease is brought under control, to some extent, and life is prolonged correspondingly. . . . streptomycin very frequently relieves acute symptoms and prepares the way for employment of other measures such as surgical therapy, which could not have been undertaken successfully without the use of streptomycin.

" . . . I have the impression that many patients are being kept alive who would have died long ago. In many of these cases death from tuberculosis will ultimately occur, because the damage done by the disease, before the drug treatment, was so severe. In other words in many cases death is simply postponed."

Dr. Long, addressing a somewhat similar inquiry to Sir Allen Daley, Health Officer of London County, England, had previously received his views, which I am privileged to relate. They follow in part:

"There was already a rapid slipping back to the pre-war mortality trend before antibiotics exercised their full effect, so they cannot be given credit for all the improvement. A whole complex of social and medical conditions have been pulling the rate down since 1850 just as infant mortality and other types of mortality have declined. At one time or another the emphasis has been on one or other element in this complex, sometimes nutrition, sometimes housing, sometimes surgery or chemotherapy—it is difficult to isolate their separate influences.

" . . . controlled studies have proved the benefit of antibiotics in selected, i.e. 'indicated,' cases, but to measure the full effect on the general tuberculosis death-rate we would need to know how many and what kinds of cases have received antibiotics. This we are never likely to know. . . . The only clear case is that of tuberculous meningitis—in 1938 the death-rate in London under 15 was 12.6 per 100,000; and in 1944, 14.1; 1945, 12.6; 1946, 13.1; 1947, 10.6; 1948, 8.42; 1949, 5.91. We do not know the total incidence because notification is incomplete, but tuberculous meningitis is a barometer of infection and its incidence has not been falling (except in 1950 perhaps). . . . It seems certain that 1947 marked the intervention of streptomycin."

Penicillin exerts a powerful spirocheticidal action when it is administered in fairly large dosages and for an adequate period of time. First used by Mahoney in 1943, it has now become the accepted and standard form of therapy for syphilis in its various forms. As reported in U. S. Public Health Reports, the incidence of syphilis dropped by more than 50 percent between 1943 and 1950 (TABLE I). The present incidence of primary and secondary syphilis is even less (TABLE II). During this same period deaths due to the disease declined from 16,263 to 11,616. How-

TABLE II  
CASES OF VENEREAL DISEASES REPORTED TO THE PUBLIC HEALTH  
SERVICE, FISCAL YEARS 1941-1950  
(Known Military Cases Are Excluded)  
Thousands of Cases

Year	Syphilis					Gonorrhea
	Primary and secondary	Early latent	Late and late latent	Congenital	Not stated	
In Continental United States						
1941	68.0	108.7	201.2	17.6	82.4	191.3
1942	75.7	116.4	202.2	16.9	61.0	212.4
1943	82.2	148.9	253.0	16.2	64.6	275.6
1944	78.4	122.4	203.4	13.6	40.4	300.6
1945	77.0	101.1	142.7	12.3	23.1	285.0
1946	95.0	107.3	125.8	12.1	20.7	364.9
1947	106.6	107.8	122.3	12.3	24.4	400.7
1948	80.5	97.7	124.0	13.3	22.6	363.0
1949	54.3	84.3	121.9	14.3	13.9	331.7
1950	32.2	65.6	113.2	13.6	6.9	304.1

ever, since these show a decline also from 1940, before the advent of penicillin and during a period when incidence was rising, it is obvious that other factors also have contributed to decreased mortality. When one considers the normal course of this disease, such factors may be understood in part, for death would be expected to occur years after the onset of the infection. The newer broad spectrum drugs are likewise highly active against the infective organisms and currently these are being used in treatment of penicillin-refractory cases. There is no question that the antibiotic drugs, particularly penicillin, have had a very marked and favorable result. As

in the case of gonorrhea, it can be argued that the means, if not the will, for eradication of these diseases are now available.

Curtis, of Ann Arbor, and collaborators from six metropolitan areas have this to say in a recent report:

"After approximately eight years of experience in treating syphilis of all types with penicillin, it is evident that penicillin therapy offers many advantages over any previous method of treatment. The most satisfactory type of penicillin now in use for the treatment of syphilis is that employing a slow absorption vehicle containing penicillin procaine in oil with 2 percent aluminum monostearate (penicillin-aluminum monostearate).

"At the present time penicillin is an effective method of treating neurosyphilis of all types.

"Penicillin is the most effective drug ever used for the prevention of congenital syphilis.

"The use of the heavy metals in the form of arsenicals or bismuth does not increase the therapeutic results of penicillin when used alone.

"Penicillin alone far surpasses any previously used anti-syphilitic remedy."

With regard to the possible complete eradication of syphilis, Dr. Joseph E. Moore, outstanding authority on venereal disease, calls attention to such influencing factors as improved transportation, population instability and unrest, economic status of countries, etc. In his Malcolm Morris Lecture (1951) at St. Mary's Hospital in London, the birthplace of penicillin, he had this to say:

"In a global sense, certain infections may be eradicated entirely in one country while they flourish in another. With syphilis, as with tuberculosis, this is improbable. . . . It is, therefore, pertinent to inquire into the possible global eradication of syphilis with the means at present to hand.

"The cost of syphilis control, on the basis of United States and Scandinavian programmes, is sufficiently high as to make it difficult to envisage their applicability in some densely populated but less prosperous countries which presently have a high rate of incidence and prevalence—e.g., China, India. . . . how best can we approach the problem on a global basis?

"Mass immunisation, if this can be developed through further medical research, is a more promising approach."

The antibiotic drugs play an increasingly important role in military medicine. In combat areas they are used to forestall the development of gas gangrene and other serious infections. Behind the lines they find wide application in the treatment of osteomyelitis and other deep-seated wound infections, in addition to the multiple uses for which they are employed in civilian practice. Most important, of course, has been the saving of untold lives. Of tremendous importance, also, has been a marked reduction in the time required to return hospitalized personnel to combat status.

Some significant figures for diseases responding to penicillin and other antibiotics have been furnished me by the Office of the Surgeon General, Department of the Army. The following are illustrative: The morbidity rate<sup>15</sup> for pneumonia (all types) has been reduced from about 12 in pre-penicillin days to 7.2 in 1950; for scarlet fever from 1.02 in 1942 to 0.1 in 1950. Regarding venereal diseases, Col. F. J. Knoblauch writes:

"The improvement in treatment of the venereal diseases is largely measured by the great decline in the non-effective rate for these diseases."

Equally significant figures have been obtained through the Office of the Surgeon General, Department of the Navy. The following statement appears in the October 1950 issue of *Statistics of Navy Medicine*:

"Infectious diseases, which formerly produced a death rate higher than our present rate for all diseases and injuries combined, now rank near the bottom of the list of causes of death, reflecting the advances made in prevention and treatment of these diseases."

Some specific examples may be cited from other issues of the same publication. For the years 1936-1939 the average annual death rate for acute peritonitis<sup>16</sup> for the Navy and Marine Corps was 6.6 per 100,000—for the war years (World War II) it was 3.0 per 100,000, and this improvement is "attributed to the widespread use of the newer drugs." Fatality rates for broncho and lobar pneumonia<sup>17</sup> declined from 7.7 per 100 cases in 1931 to 0.8 per 100

<sup>15</sup> Per 1000 strength per year.

<sup>16</sup> Statistics of Naval Medicine, January 1948, p. 6.

<sup>17</sup> *Ibid.*, June 1947, p. 6.

cases in 1945. The introduction of the sulfonamides during the '30s was the major contributing factor, while penicillin played an important role after 1943 (see also report by Keefer, p. 44).

The significant reduction in non-effective rate is well demonstrated in a comparison between 1921 and 1948, three years after World Wars I and II, respectively.<sup>18</sup> During this period the non-effective rate (per 1000 average strength) for all diseases dropped from 31.1 to 18.4; while that for acute upper respiratory infections decreased from 2.5 to 1.1, for gonococcus infections from 2.4 to 0.4, and for cellulitis and abscess infections from 1.0 to 0.4. The disease death rate in 1948 was only 0.38 per 1000 strength, or about one-seventh the death rate from disease in 1921. A substantial part of this improvement is attributed to the efficacy of the new drugs, although their effect cannot, of course, be measured apart from better preventative measures and improved patient management.

#### UNFINISHED BUSINESS

In addition to the above infections for which antibiotic drugs constitute effective therapeutic agents, there is a host of other diseases and uses for which no satisfactory antibiotic is known. Among these may be listed the following:

- Cancer in its various forms.
- Influenza and the common cold—available antibiotics minimize complications.
- Poliomyelitis.
- Measles, chickenpox, and mumps—the so-called children's diseases of viral origin.
- Fungus infections, especially systemic types.
- Proteus*, *Pseudomonas*, and some *Salmonella* infections.
- Brucellosis in domestic animals.
- Plant diseases.
- Food preservation.
- A better antibiotic than streptomycin is needed for the treatment of tuberculosis. An ideal drug would be less toxic and show less tendency to develop resistant strains.

<sup>18</sup> Statistics of Naval Medicine, August 1950, pp. 2-4.

The continued search for antibiotics to meet these needs constitutes alike the major challenges and the major opportunities for the decade ahead.

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FERMENTATION DIVISION,  
NORTHERN REGIONAL RESEARCH LABORATORY,  
PEORIA, ILLINOIS

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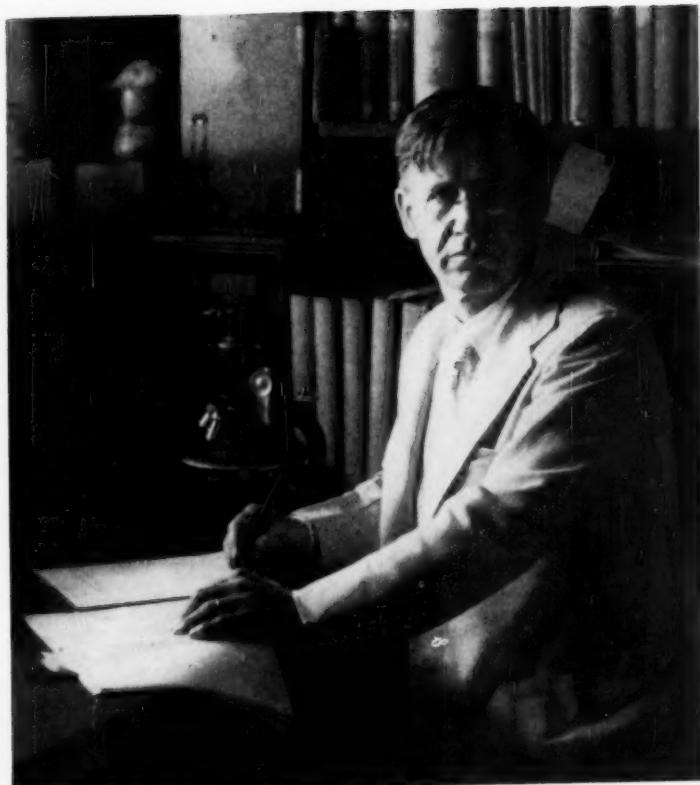
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CHARLES THOM, about 1933.

## MOLDS, MUTANTS AND MONOGRAPHERS<sup>1</sup>

CHARLES THOM

(WITH PORTRAIT)

I am acutely sensitive of the honor conferred when you asked me to give the second annual lecture of the Mycological Society of America.

Your first lecturer (Dr. B. O. Dodge) defined mycology to show the breadth of the scholarship involved and the service of that scholarship to mankind. I borrowed Dodge's manuscript and read the First Lecture. I have known him forty years—it was an autobiography of his mind. He has been a part of mycology for that period. I will follow his lead—much of what you get might be entitled *looking backward*.

The title proposed will, I think, permit me to discuss more points of contact with the thinking of more of you, than other phases of my special field.

If I may digress for a moment from the formal statements of this paper, I have stopped and looked at it occasionally and wondered. The Committeemen who selected me, know that I was retired nine years ago from the research laboratory where I had worked many years, hence in spite of wanderings in many directions since that time, would have in my head—not the organized results of current first-hand thinking, but a hodge podge of the past interspersed with the miscellaneous collections of nine years of "free lance" contacts.

### AN INDUSTRIAL MYCOLOGIST

I have been an industrial mycologist for about 47 years. It came about in this way. In February, 1904, Professor Atkinson was asked to name a dairy mycologist for a project in cheese ripen-

<sup>1</sup> Second Annual Lecture of the Mycological Society of America and address of the retiring President of the Society for Industrial Microbiology, Minneapolis, September 11, 1951.

ing. He looked around his laboratory and fixed on me to be sent, rather peremptorily offered me the nomination, then wrote a letter. I saw that letter long afterward. He wrote approximately this: "I know no man in America that is qualified in Dairy Mycology. I know nothing about it myself but if I had time, I could learn it, but I am too busy. Thom has training enough and brains enough to learn it and he needs the job." I resigned my petty but perfectly orthodox academic job at Cornell and went. They burned the bridges behind me. I never got back to academic botany.

#### INDUSTRIAL MYCOLOGY IS STILL MYCOLOGY

In the assignment of this lecture, I was definitely informed that the Mycological Society thereby repudiates any implication of lack of interest in the significance of fungi in industry and in agriculture. In other words, applied mycology is still mycology even when, in developing its problems, it uses different techniques from those of the academic laboratory. Properly developed, an industrial mold project requires a workmanship equal in quality while differing in content from classical studies of fungi. Nevertheless certain recent textbooks practically ignore the most important mycological accomplishment of the age—the development of penicillin, which has contributed much to our fundamental knowledge of fungi and their possible service to man.

#### PROJECTS

Looking backward, I have spent most of a lifetime in the mold laboratory and in projects related to molds or their utilization. It would be pleasant if I could say that it was all planned, but it was not. In each new project, if he is to get anywhere, one builds the best hypothesis that he can, from the information at hand, begins to work with the tools at hand; as fast as one theory falls before facts, he reconstructs his procedure and his mental picture.

I once saw a man facing a new project, prepare an elaborate plan covering in detail all determinations that he considered pertinent, then take a group of assistants to the field and develop data for two years. In the final report there were about 1000 pages, including one tabulation showing about 10,000 determinations. The whole was practically worthless because the plan was initially

faulty and the procedures were never altered to adjust them as the facts developed.

#### MOLDS

I engaged to talk about molds, mutants, and monographers. Perhaps I should begin by saying that for this discussion, molds are fungi, most of them styled "Imperfecti," whose visible mycelia consist of loose hyphal tangles (floccose), or more or less vertical conidiophores like fields of grain (Rasen—the Germans call them), all of which tend to break up and blow away as the spores ripen: For examples, think of the Penicillia. Very few of these species furnish stable enough specimens to be used for reference in the herbarium.

My illustrations will be mostly drawn from the Aspergilli and Penicillia with which I have done most work. If I have inferences of wider application—remember I have seen many other groups.

#### MY OWN JOB: THE TWO QUESTIONS

My own trail after molds started in the cheese laboratory, got tangled there with yeasts, Actinomycetes and bacteria, shifted to the factory, the warehouse and the market, shifted again to cattle food, to canning, to general food problems, finally back to the soil and root-rots of crop plants. Each turn brought its own technology. Nevertheless through it all, one traces the same lots of molds, and the same two environmental questions: How do they live and work in places differing so conspicuously as seen by the casual observer? And for intelligent presentation what names will take us to the literature and carry our ideas correctly to our fellow workers?

#### ANY MOLD PROJECT

My first project called for the introduction of the making and ripening of Camembert and Roquefort cheeses into the United States. Molds were already known to have something to do with it. I had never seen either of these cheeses and had never been inside of a cheese factory when I was appointed. I was told that I was to be mycologist *only*, that the chemist and the cheesemaker would carry all other phases of the project. I quickly found that *that* particular *only* was most misleading, that the one who would

develop a mold project to success must become a creative ecologist. He must *first* get the right mold, *second*, determine its capacity to do work, *third*, create the environment in which it can work best. For the actual prosecution of the cheese project, the chemist was an analyst, and the cheesemaker was a technician. The cheeses were made; you can buy them almost anywhere. That was published long ago. Some of the problems encountered will bear discussion again.

#### GETTING AN ORGANISM

*First*—Getting the desired organism. A laboratory may make broad surveys of the biochemical activity of many organisms as a means of picking out individual strains with exploitable characters. Raistrick surveyed hundreds of molds for possibly useful characteristics. Robbins and his associates have surveyed and screened a great many Basidiomycetes for antibiotic power. Many similar searches have been made in recent years.

Fleming found his penicillin-producing strain by the accident of its appearance as a contaminating organism among his bacteria but showing an activity that aroused his interest.

Waksman, searching for new antibiotics, turned to his first love the Actinomycetes, well known to all of us, as producing colonies surrounded by zones where bacteria did not grow. The result was streptomycin and related antibiotics which are now known the world over.

If one searches for a mold to do a particular job, he may make the elaborate survey which is time-consuming and expensive, or obtain known organisms with related activity as his starting point. Culture collections can today usually supply the necessary species or nearly related strains that reduce the search very greatly.

In dealing with "rule of thumb" processes, as in the case of the cheeses when we started, one simply goes to the product or the factory if it is accessible and isolates the species or strains already in use and begins his survey with the possible improvement of the agents as found. Such products are never free from contaminating organisms, hence all findings must be adequately checked.

Finally in any search for sources, the special student of the

particular group—if there is such an one—keeps, usually, the most reliable collection.

#### LABORATORY CULTURE

Assuming then that you have your mold, what shall you do with it? That takes us to laboratory culture, for we must have an abundance of mold material to run a factory. Whole books have been written about laboratory culture methods but while there may be nothing new about this discussion, there is *ample evidence in current publications that certain things still need to be emphasized.*

The early attempts at laboratory culture were directed at the task of inducing a mold to grow upon some substratum, for the sole purpose of getting material to write a taxonomist's description. Again, it was assumed, that if the thing grew, they could describe what they saw as nature's normal for the species, throw away the mass and refer the species to the substratum where it was originally obtained. The intervening substratum might, or might not, be mentioned or kept as an herbarium specimen.

Lindau, for example, showed me how he carried his lunch to the herbarium where he worked, put some of his bread in a glass dish, boiled water for his tea, poured some boiled water over the bread, placed a lot of spores in one place upon it, covered it with a glass plate and if he got a colony, had something to describe.

#### "NATURE'S TYPES"

So, depending upon the concept of nature's types, borrowed from the phanerogamic taxonomists, new species continued to be described. Color, shape of colony, and reactions noted in detail on a favorite formula were made part of a diagnostic description which was then without mention of the formula, transferred bodily to manuals or to Saccardo's Sylloge to plague subsequent workers. Several steps in the process of establishing culture as a basis for taxonomy must be discussed.

#### SEARCH FOR THE OPTIMUM

Search for the optimum culture substratum eventually lead to the publication of species descriptions on particular selected nu-

trients followed by notes about the differences obtained with other formulas. Since these common saprophytes will grow under many conditions, each laboratory tended to develop a favorite procedure upon which results were reported in routine fashion. For example, Biourge at Louvain developed and published a modified Raulin's solution agar upon which Penicillia were studied for about 40 years. Even with the formula before them, Westerdijk's Laboratory at Baarn was never able to agree with Biourge. Somewhere in the procedures misunderstandings occurred so that they never got the same results. There was an attempt at standardization but somehow it failed. I have been in both laboratories; there were contrasts in hygrometric conditions that suggest part of the trouble.

#### STANDARDIZED CULTURE

The substratum must be definite: There may be a few elemental characters even in these polymorphic molds which are unaffected or little affected by the composition of the nutrient media upon which they grow. However, conspicuous colony characters are so directly related to nutrients and other environmental conditions that punctilious standardization and description of substratum and conditions of incubation are essential if we are to base a stable nomenclature upon laboratory culture. Suppose I cite as illustration two great laboratories that I know. In one of them, I have read a hygrometer and found the relative humidity about 20%—cultures dry up quickly there. In the other, cultures dry very slowly—they stand for months in trays on shelves protected only from dust. That is only *one* factor.

#### PURE CULTURE

A culture must be *pure*: it must be one strain of one species and free from all other organisms. Some organisms will grow together with one of them submerged but affecting reactions although difficultly identifiable. In another instance one may inhibit the other and invalidate observations of either. Where two organisms grow in the same area, it is not possible to depend upon characters needed for description. Emerson has described this problem fully in a recent paper.

Is this unnecessary advice? I will illustrate. Bainier, about 1880, described *Sterigmatocystis butyracea* and preserved his type material. Examination 40 years later showed two Aspergilli, neither of which was satisfactory as a basis for identification. In Atkinson's laboratory in 1902, there was a bottle of 25% lactic acid on the table where we made cultures. We were told that bacteria are always present with mold spores and that added acid will keep them from making trouble. Such added acid is unnecessary when work is done properly but we did not know it then.

Sometime in the 1920's they got about a half dozen *Fusarium* specialists together. They got out all of their Fusaria, cultivated them, discussed them, agreed upon type cultures and sent transfers of those cultures to the American Type Culture Collection. The molds of that collection were then in charge of Dr. Margaret B. Church in my Laboratory. She examined them as received, brought them to me, rejected the whole lot and sent them back because they were so generally infested with mites and bacteria that no dependence could be placed upon them. They went back to the designated custodian of Fusaria who insisted they were in proper condition. I had received other cultures from that man's laboratory and in each case, they carried mites and bacteria. I think he must have depended entirely on slanted tubes, hence was never able to examine the surface of his mold colony (as in the petri dish) with the compound microscope and lacked the curiosity to make free use of a good hand lens.

There are whole series of papers in certain fields today in which no claim of controlled culture is even suggested. Forty years ago we would have believed that these organisms could not be induced to grow in artificial media—today, I do not believe it. I believe a lot of that work will need to be repeated before we really know those species. I do not like to throw mud at the idols but I believe that there are great series of pathogens which call for reinvestigation if their names and descriptions are to mean anything.

#### MITES ET AL.

Other similar experiences forced me to recognize that there are mycologists who do not know mites and their depredations when

they see them and did not find proof of their travel from tube to tube in the bacterial slimes that mark their trail. One group about ten years ago asked me to look at their trouble in which the surface of their mixture looked like the surface of a well-smoothed hog wallow: examination of the sides of the vessel, as much as 2 inches above the slime showed thousands of nematodes. They had not even used a hand lens or perhaps would not have known that the nematodes did not belong there.

I have searched certain recent papers for evidence of purity of culture, or some assurance that the organisms discussed can be obtained in some particular manner, but find no help. The warning is needed.

#### ACCIDENTAL MIXTURE

In the field, molds grow in accidental mixture usually with several organisms.

The colony we find in outdoor mixed-culture is the expression of the capacity of the mold itself to grow under the combined influence of the nutrient supply and the competing organisms. Cutter's gang calls it a paramorph. The same mold may present a different aspect when next encountered, if the environment is different. There is only one satisfactory way. Get a "pure" culture before you start naming molds, with the added corollary, check that purity on every strain of mold, no matter who sends it to you, before you start research with *it*, as an agent.

#### STRAIN

Right here, avoiding corrupting such words as the geneticist uses, I will call the pure culture that I have advised you to get—a strain—a colony of a single species which we are ready to use in the factory, to subject to a biochemical study, or to try to allot a Latin name as a member of some taxonomic group. For the purposes of this lecture I will now leave the factory and the biochemical laboratory and go to the problems of nomenclature.

#### EARLY MYCOLOGY: MICROSCOPY

The early mycologists were microscopists primarily. They examined fresh or dried material under selected magnifications (often

unspecified) of whatever microscopes they had. They wrote up what they saw in Latin as species diagnoses. Three lines were long descriptions; few got that much. They attached Latin names and let the next generation try to identify specimens. Their original specimens promptly fell to powder in the herbarium.

Micheli, Haller, Persoon, Link, Fries, Corda and the rest were pioneers. They were intent upon reporting nature as they found it. Sometimes one is tempted to add—as soon as they found it. There was little background of information because the compound microscope was a new tool. Those of us who have tried to put a sound nomenclatorial background under the newer mycology have gone over their books line by line. Much of the work they did is still valid. But in the area that I have worked, I regret to say, we must just go away and leave them.

#### THE SPECIES MUDDLE BEFORE 1900

This nomenclature problem may be illustrated from my own project. It did not take long to isolate the cheese molds. My microscope told me they were Penicillia. (I had seen one *once* in college.) But while I was getting these I had found perhaps a score more which manifestly belonged with them. I turned first to the cheese literature. The French and German workers used Latin names freely. Only they did not agree in their usages. They gave no descriptions. There was no choice but to start back toward original authors. Again I got no help; my two species would not satisfy the diagnosis of any historic species. A dozen or so more of Penicillia were also growing before me. Thus I was driven to monography.

#### THE MONOGRAPHER FACES 100 YEARS OF SPECIES

Elsewhere I have written—"the monographer worships his ancestors." His task requires him to find out everything the describer of each species in the group has said about it, and if possible find out exactly what organism he had when he wrote the description. The whole story should appear in the monograph. I worked the "classics" over line by line. When I got to the early 1850's, I read Montagne's comment that no one could at that time identify,

from the description, a single *Penicillium* described before his period. I agreed and regretfully add that the first *Penicillium* described and accompanied by material which proves its identity was *P. digitatum* of Saccardo about 1881. By combining Link's description (1809) and his vernacular notes with my survey of rotting fruit in Berlin about 100 years after Link had described it, Dr. Shear and I used *P. expansum* Link as type species of the genus.

In that 100 years, a lot of names were published for species of *Penicillium*. I have read every one of them. Just four of them can be tied closely enough to material to mean much today. Two of them have been discussed already. One of the four, *P. italicum* of Wehmer, I saw in Wehmer's laboratory in Hanover. He took me to the window, pointed to a fruit stand, and told me to go and get an orange with a blue green rot and verify it with the microscope against his description. I did as I was told and got his idea.

In the naming of the fungi, certain fundamental assumptions had been borrowed from phanerogamic taxonomy.

#### THE TYPE SPECIMEN

(1) The specimen first found and described was always to be regarded as type for a valid description. Any marked deviation justified another species or varietal description. No description from artificial culture was valid. The type so designated was nature's type.

#### LOCALITY

(2) The place of finding the original specimen became the habitat of the species. Westling obtained the original colony of his *Penicillium notatum* from a dilution culture from leaves of hyssop found moldy in his pharmacy. Hence hyssop was referred to as the proper place to look for *P. notatum*! (We were in the 1940's.) The thing grows in hundreds of natural situations. When the first finding of a fungus was in a *dilution culture*, hence without identifiable specimen, they dubiously accepted the colony in the culture but referred the habitat back to the mass diluted; again the seeker for information is helpless. We were up against the fiction of nature's type. This reappeared in a recent paper as "wild types."

## THE FICTION OF A NATURE'S TYPE OF MOLD

In handling the evanescent saprophytic molds, it became necessary to get rid of the fiction of *nature's types* as applied to these groups and to destroy any emphasis on the place of first discovery as a significant habitat. I have already pointed out that the colony found in nature so far as these groups are considered is just *the one paramorph* of the species that *happened* to be first found. As a *nature's type*, it is no more normal as a representative of the species than any other colony produced in relation to a different environment. The term "wild type" is equally a misnomer. Nature did not furnish us with a *type*.

## WE MUST MAKE OUR TYPES

If then we are to describe these molds and to have a type for later use in comparative study, we must *make* it. I am more or less responsible for this dictum. I lived with the bacteriologists and absorbed some ideas. The *starting-point* is a *pure culture* as already fully discussed. The *next requirement* is a *nutrient substratum for descriptive work*. This must satisfy certain demands: (1) Components of the formula must be chemical substances purchasable in standard strength from any supply house anywhere. (2) It should be definite and readily compounded in any laboratory "media" room. (3) The concentrations used must support a reasonably good growth from many species rather than the optimum growth of single species or strains. (4) Descriptions of organisms grown upon such a substratum should *cite the culture medium in the first line*. (5) Such type cultures should be contributed to culture collections and stored in such manner as to retain viability and morphologic identity.

## DESCRIBING PENICILLIA

For the Penicillia, I selected the neutral form of Dox's modification of Czapek's solution. This is commonly cited Czapek-Dox, especially by those who use it in acid form. I used  $K_2HPO_4$ ; the acid form is  $KH_2PO_4$ . The other components were unchanged. The continental mycologists, at least some of them, still use *beer wort* which gives heavier growth and more abundant spore forma-

tion. In dealing with *Penicillia* and *Aspergilli* the Czapek formula gets rid of the uncertainties in composition of the beer wort and reduces the mass of growth without sacrificing the development of structural details. Even among these forms, an occasional species refuses to grow, hence must be specially handled.

A form of species description was gradually developed upon Czapek supported by line drawings. A series of them, made to test out the idea, was put before Professor Klebs in Halle. He listened more or less tolerantly at first, then burst out, "Gut! Aber die Grenze?" Under argument he finally admitted that a standardized description might be supported by comments without the incorporation of all extremes of variation due to nutrients into the diagnosis. The source and distribution of a species or strain in nature is often pertinent information.

#### THE MATERIALS FOR A MONOGRAPH

Preliminary work over several years thus finally produced the raw material for a monograph. This includes: (a) organisms in accessible condition (type cultures), (b) a form of description, and (c) a complete bibliographic survey of the genus. Testing over several years seemed to justify the belief that such a description could be used to identify a mold received from unknown sources. Other experiments seemed to show that a mold could be taken from stock culture, used for numerous experiments, after which it would resume the aspect represented in the description when returned to the standard substratum. Meanwhile hundreds of strains of *Penicillium* had accumulated in the collection. From the responses of certain species, we believed we were justified in going forward with the monograph.

#### NOT A GENETICIST; NOT A CYTOLOGIST; A MORPHOLOGIST

A monographer is not necessarily a geneticist and not a cytologist however familiar he may be with their work. He serves the worker seeking identification of material in hand. The allocation of an unknown mold to a particular place in a taxonomic system must be based upon morphology in the inclusive sense. He must specify for the purpose, characters discernible with the naked eye, the

hand lens or routine use of the compound microscope. Routine culture is necessary in handling these molds. A satisfactory description should enable a worker with routine knowledge of the morphologic terms used in the group to obtain an identification. E. A. Walker, dealing with flowering plants, once wrote: "A description that forces the user to consult the original specimen, has indeed defied its very purpose."

#### THE AGGREGATE CONCEPT

And we still believed in species. Then we started to put it all together. It was not difficult to select out great series in which relationship was easily apparent. To key these series was comparatively easy. A few sharply defined species stood out conspicuously and fixed particular points in a taxonomic system. Then great series of strains with many points in common were grown in petri dishes and spread out for comparison. Some of them presented more or less contrasts which continued to be discernible in consecutive transfers but when considerable numbers were compared, the spaces between strains were no longer conspicuous. Some method of handling these series was needed.

For the Penicillia and Aspergilli, we developed the concept "aggregate" species for those series in which there is essential agreement in morphology and in the nature of reactions to environment even though quantitative differences may at times be rather striking if we compare members separated in the series. This threw together, for example, a half-dozen black Aspergilli of Mosseray's series differing in their color production in the substratum, and again for another example large numbers of fruit rot strains widely spaced in *Penicillium expansum*. From many comparative studies in the laboratory and ample factory experiences with penicillin production, it is concluded that the members of such series participate in the same kind of activities but may differ enormously in the quantitative expression of them. Among different members of the penicillin producing series, some of the best ones produce as much as 50 times the amount produced by some of the poorer ones. Many such strains retain their relative productivity in successive transfers. Nevertheless in seeking for an

identification, assignment to such a series of closely related strains seems more useful than any attempt at separate description and nomenclature.

#### FACTORY NEEDS: A DEPENDABLE STRAIN

Many of those conclusions were developed from the needs of industry. Hence some of the problems encountered may be discussed. The factory wants a dependable strain, and the highest yielding strain available. In the hands of the technician, strains at first productive often became worthless in a short time with resulting losses of material and time. We come thus to the third item in our title—variation, mutation or what is it?

#### ONE BIOCHEMICAL ACTIVITY SOUGHT

An industrial fermentation process calls for the maximum possible development of some single or as few as possible phases of the biochemical capacity of a fermenting agent. Every other normal activity is suppressed, as far as possible, as a useless waste of the operator's material. For this purpose the laboratory investigations (Raper *et al.*) concerning *P. notatum* have been productive. *Penicillium notatum* in surface culture begins to produce green areas of spore production within 2 or 3 days. Such spore production normally uses up much of the metabolic product of the mold. In a penicillin tank, the operator wants usually colorless shreds and almost microscopic clumps of vegetative mycelium which is kept in active motion coming in contact with every cubic millimeter of many thousand gallons of a liquid selected because it favors a single activity—penicillin production. It is not a balanced ration. The resulting paramorph has no taxonomic value although it has earned millions of dollars.

The inoculum for such a process must be true to type, and must be at the point of development at which it is capable of the utmost activity. Spore production is suppressed as completely as possible and at the end of the process, the mass of mycelium is hardly capable of profitable use over again for the same purpose. The story of other fermentations differs in procedure but ends in the rejection of the mass of growth developed as unfit for the next inoculum. The next fill of the tank must have a fresh culture.

## FAILURE OF THE STRAIN IN THE FACTORY

Failure of stock cultures to maintain penicillin-producing power started extensive studies in two general directions; first, procedures which would maintain a strain without this lost value, and second, what happened. This led also in two directions—(1) the search for new strains from nature, and (2) induced variations in the best strains already in culture.

## STABILITY IN STOCK CULTURES

Some procedure that will maintain strains unchanged for long periods is important for the laboratory collection as well as for the factory. We had many well-known species whose type strains had remained satisfactorily in the collection for many years. Some had been lost in spite of efforts to keep them. Others had assumed quite different aspects from that described when the name was published. Some showed *sectoring*, the appearance of contrasting structures or colors in the same colony. Some ascosporic strains had dropped and refused to resume the ascosporic form.

## MASS TRANSFER

To satisfy the demand that a strain kept in successive transfers should survive without alteration of aspect or change in its biochemical activity, closely checked experiments proved that the transfer of large numbers of spores, taken from typically sporing areas, to fresh culture media would produce dependable colonies. To maintain a static culture, this procedure is practical in the hands of the technician and equally useful where the keeper of a collection needs to be able to maintain a special culture. As an example, Steinberg, closing his work at Columbia University, sent his black *Aspergillus* to me. Nearly twenty years later he asked for its return for use in new work. He reported that rechecking it in fresh culture verified its identity before new work was undertaken. Certain cheese molds were kept satisfactorily for more than thirty years.

## SINGLE SPORE TRANSFER

Demonstration of the practical usefulness of mass transfer left no analysis of the problem. Zaleski made a special trip to my

laboratory to tell me that my practices were all wrong and my collection would continue to be worthless until every culture was based upon the transfer of one spore—hence undeniably pure. In their study of *P. notatum*, Raper *et al.* tried it with rather surprising results. Among a hundred or more of the resulting colonies "screened" for penicillin production, there were variations in colony aspect, as also in yield of penicillin. A few were outstandingly good producers, the remainder varied down to the extremely poor. Single spore culture, it was concluded, proved to be one way to obtain a small percentage of new and valuable strains but a failure in the maintenance of stability in known strains.

#### ANASTOMOSIS

If we go back to the significance of mass transfer after noting the variability among colonies derived from single spores, we have overlooked a striking microscopic feature. Careful examination of the colony developing from several to several hundred of spores shows that the mycelium so developing is not simply a lot of interlacing hyphae each stemming from a single spore but a mat of anastomosing hyphae. Such a colony then is not a closely woven lot of individuals but essentially an organism, perhaps it might be called a blend. Please note I have left out all genetic terminology here—the thing *works*. Miss Baker has pictured for us, multi-nucleate cells with dividing nuclei floating back and forth but offered no real explanation of the phenomenon of anastomosis.

#### A GUESS AT WHY

So far as reported, cytological studies have not explained anastomosis. A rough generalization applicable to Penicillia may be hypothetical but perhaps suggestive. The chain of spores (conidia) attached to a single basal cell or sterigma may show 100 or more individuals; each of these (the oldest is the one farthest away), was derived directly from the sterigma. In that cell, in most Penicillia which are one-nucleate, the nucleus divides; one daughter nucleus migrates into the developing spore forming tube; the outer segment containing the new nucleus is cut off as a new cell; the lengthening tube pushes the first cell outward and the process is

repeated. All of the spore nuclei are daughter nuclei of the sterigma nucleus. Although they may all remain attached in the chain, the newest cell is directly upon the sterigma. There is no cell division in the chain. Should they all be alike? I've looked at thousands of them. If the average diameter is  $3.5\ \mu$ , there will be some  $3\ \mu$  and some as much as  $4\ \mu$ . If planted separately, the colony variations will fall among those well known for the strain. One may infer that the inheritance (nuclear) of morphology is sound. The difference in biochemical activity may perhaps be cytoplasmic and we have no way of knowing what "precautions" the cell took to divide that equally.

I am quite aware of Miss Haenicke's and Miss Baker's cytological papers on the Aspergillaceae. I explored Miss Baker's method of germinating spores on the slide many years ago and discarded it because I could not depend upon any morphological data obtained. Her nuclear studies remain suggestive. She has suggested the possibility that the multinucleate stalk of an *Aspergillus* furnishes a whole regiment of nuclei which migrate first into the vesicle then through the sterigmate cell into the successive spores formed at its apex thus supplying nuclei to the spores from the mycelium (?). The *Aspergillus* head thus becomes a mechanism for the distribution of nuclei which have their origin in the foot cell and stalk of the conidial apparatus. I have looked at more thousands of them than she has described individual specimens and attribute some of her observations to the abnormalities of her method of growing material. The foot cell and conidiophore of every *Aspergillus* is, in my judgment, a specialized apparatus for maintaining the species and well segregated for that purpose.

#### SOME PROBLEMS OF NAMING

Thus when we tried to get some order in our observations of these delicate but persistent and almost omnivorous molds, we developed a lot of problems which I have tried to describe here. Let me then summarize our progress, perhaps here and there pointing to work yet to be done. Some few of these have close relatives that are ascospore producers. Those are only a routine problem to the monographer. I have barely referred to them

since they have been appropriated quite generally by the geneticists. Our problems concern so-called imperfect fungi that are grudgingly allowed names. Our most conspicuous example is *Monilia sitophila*, which was well known only as a pest for about 60 years or until two or three of us in widely separated places picked up the ascospore form. They were good enough to us, to call that *Neurospora* and leave the old name *Monilia sitophila* to the miscellaneous lot of conidial molds which in the states north of Texas constitute 99.9% of the molds of that series which one meets in general surveys. True, perhaps, they are all haplotypes; true also that no one can ever guess which one Montagne had when he proposed the name. Nevertheless they abducted the name "*sitophila*" for one ascospore producer.

#### "SHOTGUN" CULTURE MEDIA VS. ANALYSABLE AND ADEQUATE MEDIA

I have limited most of my comments to Penicillia and Aspergilli, which were my avocation for about 40 years, but the problems encountered there apply in greater or less degree to other mold series which I have been compelled to handle, by the official problems of the laboratory. The bio-chemists and physiologists working intensively on special problems have made available and even adjusted to laboratory routine, nutrient substrata of exact composition to replace the "hit and miss" "shotgun" mixtures devised a half-century ago and still in use in many places. Fungi that simply refused to grow upon the messes that we offered them, are now readily cultivated. Even some that we called "obligate parasites" grow freely in the laboratory today. There remains little excuse for the special study of morphology and life history in mixed cultures upon unanalysed hence unrepeatable nutrients. We know too much about the influence of chemical composition of nutrients and other environmental factors to be satisfied with what we get under uncontrolled culture conditions.

#### MUTANTS, VARIANTS, ET AL.

So far, I have nearly if not quite kept away from the term, mutants. During the later stages of development of our monographs, occasional weird forms of Aspergilli began to appear to

disturb our thinking. One of them was so striking that Yuill called it a new genus *Cladosarum*. Study of it in the laboratory convinced us that it could not propagate itself readily in competitive environments, hence could hardly become a member of any natural flora. It was therefore noted only as an anomaly not included as a genus in the Manual. About the same time, Mosseray, studying 63 strains of "black" Aspergilli, decided upon 40 of them as separate species, many of them undescribed. We had perhaps an equal number of variants only partly coinciding with Mosseray's. Meantime Steinberg, as noted already, had reclaimed his strain which, by Columbia University tradition, had been brought from Paris by H. M. Richards and was supposed to trace back to Van Tieghem. It had held the same aspect for 20 years with us. He then began his series of stimulation experiments with known chemical compounds. He picked out all the weird shapes that he found and passed them over to me. Without reciting details, the old respectable *A. niger* T and C 4947, under metabolic stress, produced the same general types of variants or mutants that Biourge's laboratory at Louvain and ours at Washington had accumulated from miscellaneous and undescribed sources. These were carried readily in culture without loss of character. Mosseray then moved to Brussels where he received large collections from the Belgian Congo, among them more mutants of the black Aspergilli. He sent them all to me.

#### MUTANTS AS SPECIES

Certain of these mutants, recognizable as belonging here but presenting definite morphological characters, have been included in our manual. We are convinced that many widely distributed species began as mutants whose antecedent species are sometimes recognizable. If such a mutant is an efficient producer of viable spores, and is capable of growth on a wide range of substrata, it can be expected to survive. Among the black Aspergilli, nearly all of the mutants encountered are readily recognizable as belonging with the *A. niger* group. Most of those seen are extremely rare, with irregularity of structure suggestive of defective units, and give the impression of arising where found. They nowhere

appear as large colonies. From the monographer's point of view today, they are best referred to the aggregate series until some one on the basis of intensive study decides upon proper disposal.

#### CLADOSARUM FOR EXAMPLE

Yuill's mutant "*Cladosarum*" calls for some discussion. In their paper they gave ample photographs but did not interpret the morphology shown. We have grown it and gone over it carefully. As already discussed, in most of the Aspergilli and Penicillia, the nucleus of the basal cell or sterigma actively divides and one of the resulting nuclei passes into the spore-forming tube to form the nucleus of a spore and to remain inactive until the spore is in a favorable place to grow. In that way, spore chains like strings of beads result. The chain lengthens only by the production of new spores at the end attached to the sterigma. In *Cladosarum*, the story is reversed. The inactive nucleus remains in the sterigma, the active nucleus migrates to produce a sterigma-like cell, not a spore. The basis of the mutation appears to have been the inversion of the original nucleus of the sterigma. In the chain resulting, the active nucleus always migrating to the tip of the chain, produces not spores but replicas of the basal cell, sometimes producing clusters of several secondary basal cells each of which produces a new branch of a complex brush made up of branching chains. Then we remembered a fantastic thing found by Barnes (1928) when he heated some members of the *Aspergillus glaucus* group. When we restudied that, it was seen to be the same type of mutant. Apart from carefully protected culture only now and then would startling mutants such as these survive. Dr. Raper informs me that several such mutants have appeared among recent induced variants of the Peoria laboratory.

I am quite aware that Haenicke reported several nuclei in the conidia of *Aspergillus carbonaceus* and some other large-spored forms. The cytologists do not seem to have settled all the nuclear questions here. Morphologically the only essential difference in the structures found, is size. Whether the multi-nucleate spores in the large conidia of *A. carbonarius* or *A. fonscecaei* are migrants, or daughter nuclei of an original one-nucleate cell, we can leave

to the Cytologist—for the monographer, the spore is 2 to 3 times the original *A. niger* spore in diameter and dependably so.

#### INDUCED MUTATION

Thousands of mutant strains produced by irradiation or chemical stimulation of known cultures have been "screened" for antibiotic production, and for exact physiological studies. The yield of penicillin has been greatly increased. Some strains have been obtained which lack particular enzymes, or produce other enzymes in much larger quantity, or which lack the ability to produce particular needed vitamins. These are now the well known tools of particular laboratories. Genetic analyses report interesting stories. But our question is, from the monographer's viewpoint, have these investigations produced organisms that can be or should be described as species? What would they do if massive inoculations of them were distributed to the farm, the forest, or the swamp. They succeed in pure culture, but pure culture is man's device intended to separate an organism from the competitive turmoil of its accidental or ecological association so that we can see it, measure it, photograph and describe it. The gap between the monographer's materials and those of the genetic laboratory is not so wide as it once was. However, until an organism can be found as part of some flora and separated from related organisms by ordinary descriptive terms, it is doubtful if one need give it a Latin name and include it in a monograph. It can, however, be a valued strain in a collection or treasured for its activity in some laboratory.

#### EXPLOITED AND EXPLOITABLE STRAINS

Why all this discussion? Knowledge of the importance of the common molds in man's affairs has been growing rapidly. The development of Penicillin gave a tremendous impetus to studies which had long been quietly in progress. Commercial laboratories have been intensely interested in developing exploitable products of mold activity. Men suddenly began to understand that there is an intensive struggle for existence among microorganisms wherever rotting or fermenting processes are active. It was a short

step to the idea that every organism which survives in this conflict, is somehow specially armed to succeed. Some have firm, almost impenetrable walls. Some attack carbohydrate and surround themselves with zones of intense acidity; others produce substances which are toxic or otherwise cripple competing species. A single group, the Actinomycetes, seem to produce a different antibiotic for each species or even variant. The Penicillia and Aspergilli are mostly armed to get a fair share of almost any nutrient substratum, while they thrust their fruiting hyphae into the air above the microbial turmoil in the medium and release countless spores to be wafted in every direction.

#### WHAT SHALL WE DO?

I have tangled with plant parasites, mushrooms, slime molds, water molds, with miscellaneous rots which perplex us in soil studies, destroy our stored foods, disintegrate our fabrics and even attack our bodies. I honor the pioneers who laid the best foundations that they could, with what tools they had, and set us to thinking. I have no cavil with the mycologist who, following the advice that Whetzel gave him at the Richmond meeting, goes to the field and "lies on his belly in the swamp to study nature with a hand lens." But after he has seen what he can in the swamp, I challenge him to bring it in and find where it fits into some scheme of things before he bursts into print about it. I lose patience today with the man who working with some concrete taxonomic group publishes 1 to 3 new species about 3 times a year for 10 years without straightening out the mess himself. I suppose there was a time when men held their breath to hear of new species, but that was long ago; today we need coordinated study. Such considerations drove me to monography with forty years of collecting, culturing, comparing, and the bibliographic explorations involved.

I have then presented some of the mental meanderings of a mycologist who was thrust into a field at that time (1904) neglected, and in which the landmarks were few and mostly misleading. Like the bacteriologists associated in related tasks, I had to depend upon the culture laboratory instead of the herbarium for

my reference material. Having to deal with fungi unrecognizable by orthodox methods, there was no choice but to find my own way of doing it and insist upon its recognition.

There is a new literature based upon the culture laboratory in which a great array of single species papers, differing widely in their viewpoints, present new problems of coordination of the old and new.

The morphologist, the cytologist, and the geneticist go their several ways and often leave the homogenizing job undone.

In a recent survey of research publication problems (*Science* 114: 8/3/1951, p. 134) we are told that about 750,000 papers each year are published. Perhaps 250,000 of them are abstracted in some language. Hence it is proposed that professional groups of readers should be established to marshall the ideas covered in such form as to keep the "bench scientists" working profitably. Here is an experience with one of such resulting editorial prodigies. He never did a "bench" job. He got the chairmanship of the production of a great book to combine the results of research in a broad field. He assigned my office approximately 8 pages. Later, an instructional letter commanded the inclusion of certain paragraphs prepared for us by his office. Unfortunately, those paragraphs were incorrect as to the facts. There is probably much information in that book—I have read in it, here and there—but I would not dare trust it to answer a critical question while most of what I find apparently endeavors to follow the dictum once given to me as a beginner by a chief, "don't tell any lies you will get caught at." In other words, it is vaguely general.

In spite of this prodigious volume of publication, the world of critical scholarship in any field is small. If there is any single message that I wish to pass along, it is that a man who has long done a really scholarly job in any special field should stop his stream of paltry items and make a monographic study of his special research territory at least once in a lifetime or better once in every ten years. His fellows are entitled not to the printing of his notebook, not to a rehash of his contributions over a period, but to his critical judgment of what it all means when brought together with the whole literature of that field of thought. Such publications

would be a larger service to his fellows than a few more petty contributions. A great task is never finished. To my mind, monographs to date are an obligation.

#### SUMMARY PARAGRAPHS

The industrial mycologist is an ecologist who first finds an organism which can do some desirable thing, then creates the conditions under which the organism is best able to do the particular task. If he is to be anything much beyond a laboratory technician, he must identify his organism as a unit in some taxonomic group and compare related molds.

In the industrial laboratory using mold activities, there is no nature's type for his molds. If the mycologist is to have a dependable type, he must create it and care for it in culture.

A type culture must be a pure culture. All of the other molds, bacteria, yeasts, or other organisms which come into the laboratory with it must be eliminated.

For an industrial process the optimum procedure is the one by which the utmost quantitative expression of a desired metabolic activity of the mold is developed, while as many of its other activities as possible are suppressed. The optimum for one special purpose will be different from that necessary for another activity of the same species. The mass of mycelium produced may be useless for general purposes.

For maintenance and taxonomic description the substratum used should be one upon which many members of the same genus of molds will produce good colonies for comparative morphological study, and one which can be made up in any laboratory from standard chemical materials.

In a description of a mold from culture the substratum used should be accurately designated in the first line of the description.

For a taxonomic comparison large numbers of strains belonging to the genus must be brought together and grown in comparative culture under as nearly as possible uniform conditions. Under such a program, the strains of *Aspergilli* and *Penicillia* quickly fall into great series of related, when not identical, organisms. Variation in detail is the usual presentation, not identity.

The lot of related strains breaks again into series, the members of which are so closely related in characters that they seem to grade together. To such series we (Thom and Raper) have used the term aggregate species. Even though minor differences may be maintained in successive transfers, the resemblances outweigh the differences and justify naming as members of one species.

Variations in the quantitative expression of a biochemical reaction, even though stable in successive transfers, do not justify more than varietal status.

Variations of a qualitative nature—presence or absence of activities, definite morphological differences that are carried stably in successive transfers—may justify species designation, whether arising from known or unknown stimuli. An induced mutation may thus justify a species description if it produces a well rounded unit, or may remain a laboratory curiosity or a biochemical tool maintained only by special cultural means.

## TWO NOTEWORTHY SPECIES OF SEPEDONIUM

SAMUEL C. DAMON

(WITH 1 FIGURE)

Although the species discussed here are not, I believe, uncommon, they are poorly known and generally neglected. Both have been reported before, and one of them is now represented in the culture collection at Baarn, Holland, but its essential features have not been emphasized. Both species probably have a world-wide distribution.

During the course of a taxonomic study of *Sepedonium*, isolations were made which were similar in general appearance to *Sepedonium chrysospermum* Link ex Fries in all respects except their conidial form. The same fungus has been reported only once before, in a report on Brazilian fungi by Viegas (10) who included it as *S. chrysospermum*. His description and illustrations are extremely clear and leave little doubt concerning the identity of his fungus, and the complete identity with the isolations described here was confirmed after comparison with a specimen kindly sent to me by Dr. Viegas.

It should be remembered that the globose, tuberculate spores upon which *Sepedonium* is based, and which come to mind in connection with *S. chrysospermum*, are not conidia but aleuriospores (Vuillemin, 11; Mason, 3), and it is the color of these spores which is responsible for the appearance of boletes and agarics parasitized by this species. Microscopic examination of fresh collections of *S. chrysospermum* reveals large numbers of aleuriospores and few if any conidia, which are also known for this fungus. The conidia are found in numbers in young specimens or in young colonies when the organism is grown on artificial media, and it is because of their inconspicuousness in mature specimens that they are usually overlooked. There is accumulating evidence to substantiate the observation that there is more than one conidial form having

aleuriospores referable to what is usually called *S. chrysospermum*, and that identification cannot be made without reference to isolations on artificial media in which the conidial forms can be easily studied. It is usually impossible to study the conidia in specimens brought in from the field because growth has progressed so far that conidia are obscured. Furthermore, in only one type are the conidia characteristic enough to allow identification when they have been found.

The conidial form commonly attributed to *S. chrysospermum* produces erect or ascending, more or less verticillately branched conidiophores having conidia abstracted from the tips of the branches. This type has been described most recently by Howell (2) and, for convenience, is referred to as the *Verticillium*-type. The conidial form isolated from *Boletus bicolor* Pk., which I collected in Rhode Island in 1947, from other material collected by Dr. Walter H. Snell, and which has been reported by Viegas and observed in material received from the Centraalbureau voor Schimmelmicultures, is entirely different. As with the common type of *S. chrysospermum* (*Verticillium*-type), the conidia are found only in young cultures. The conidiophores are erect or ascending and are little different from the assimilative hyphae in general appearance. They are usually several times branched with each branch terminating in a group of candelabra-like phialides (FIG. 1, A). The tips of these phialides are slightly flared and the conidia are borne singly or in succession. It is believed that ecological factors influence conidial production so that under rather dry conditions those produced fall away at maturity, whereas those produced under moist conditions prevailing in culture dishes may be seen floating in a drop of liquid at the tip of the phialide. When this species is grown on liquid media it has been noted that the typical conidia are not produced at all, or only rarely, and are replaced by an ovate type. Such variation can be confusing from a taxonomic point of view, but it has been my experience that at least a few typical conidia can usually be found under all conditions. The conidia referred to as "typical" are bottle-shaped, having a broad truncate base and a slightly larger central diameter which tapers to a knobbed apex. These conidia germinate by the production of

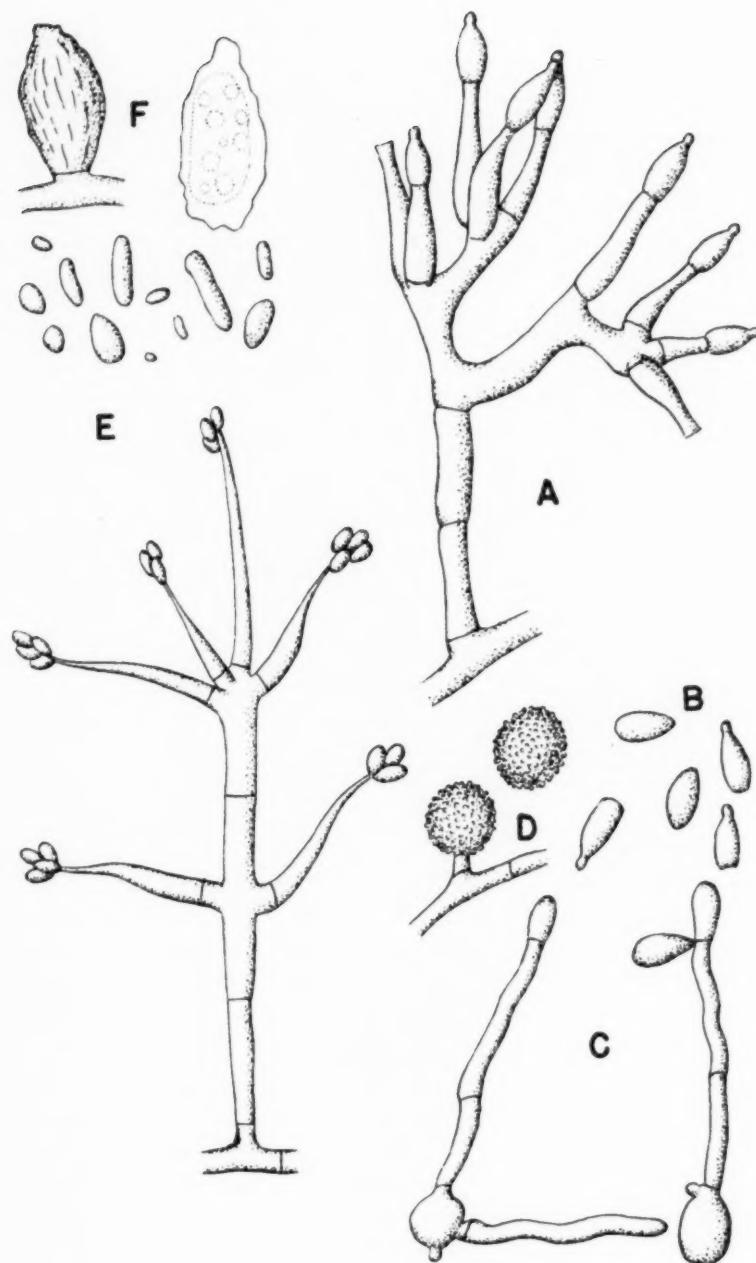


FIG. 1.

germ tubes as well as by the formation of secondary conidia (FIG. 1, C). The knobbed type of conidium described here is unique among the hyphomycetes and cannot be confused with the conidia of any other genus. Ovoid conidia are also produced in some cultures and are found almost exclusively in cultures grown on liquid media. It has been my experience that the ovate conidia tend to increase in direct proportion with increased moisture in the atmosphere whereas typical conidia are produced under conditions of low moisture. Ovate conidia are also found (FIG. 1, B) more often than typical ones in fresh material. It should also be remarked in connection with the production of ovoid conidia that they arise from conidiophores which are not branched in a typical candelabra-like fashion referred to above but upon irregular and much attenuated branches. The aleuriospores produced by this fungus are globose or subglobose, rough-tuberculate, and golden in color (FIG. 1, D) as are those of the *Verticillium*-type of *S. chrysospermum*, and their formation is the same as that described by Howell (2) for that form.

The colonial growth of this fungus on artificial media is extremely variable. On malt agar, one isolation will develop only a slight aerial mycelium while another will produce a very extensive one; and it has been noted in each of my isolations that the character of the growth remains the same through many transfers on the same medium. In all, however, the same general pattern of growth is followed. At first, it is white and floccose with conidia produced in large numbers; but after the first week the golden color, indicating the maturation of aleuriospores, appears in the center and gradually spreads over the colony until at maturity the white mycelium has almost completely disappeared and the colony has taken on a farinaceous appearance as the result of the large number of aleuriospores formed. It has also been noted that aleuriospore production falls off after many transfers on artificial media and the white aerial mycelium becomes persistent. The aerial hyphae usually remain hyaline, but submerged hyphae often become the same golden color as the aleuriospores and this color diffuses into the

FIG. 1. A-D. *Sepedonium ampullosporum*. A. Branch of conidiophore. B. Conidia. C. Germination of conidia. D. Aleuriospores. E-F. *S. chlorinum*. E. Conidiophore and conidium. F. Aleuriospores. All approximately  $\times 1000$ .

medium. In age, it has been observed that both aerial and submerged hyphae become heavily encrusted, although this property may also disappear after long periods on artificial media.

Early attempts to identify the isolations reported here led me to assign them to *S. macrosporum* Sacc. & Cav. on the basis of the wider range of variation in the measurements of the aleuriospores. The workers at the Centraalbureau, in identifying the culture of an identical organism deposited in their collection, must have followed somewhat the same reasoning except that they have assigned the name species rank whereas I was inclined to follow Ferraris (1) in considering it only a variety of *S. chrysospermum*. Aleuriospore morphology, aleuriospore measurements, and general similarity to *S. chrysospermum* indicated only varietal rank for the name. This seemed to be borne out by the results of a parallel study which yielded a large number of aleuriospore measurements of both *S. chrysospermum* and the isolations presently referred to *S. macrosporum*. The following table presents the measurement data of 400 spores and their statistical analysis:

	<i>S. chrysospermum</i> ( <i>Verticillium</i> -type)	" <i>S. macrosporum</i> "
$\bar{x}$	16.0 $\mu$	17.22 $\mu$
$\sigma_x$	2.04 $\mu$	2.5 $\mu$
range ( $3\sigma_x$ )	9.88-22.12 $\mu$	9.72-24.72 $\mu$
range (by direct measurement)	11.0-22.0 $\mu$	11.0-24.0 $\mu$

The difference of the means was tested and was not found to be significant. It may be concluded, therefore, that although there appears to be a slightly higher mean value for "*S. macrosporum*," this difference is statistically insignificant. Although these figures do not in any way refer to *S. macrosporum* as that organism was known to Saccardo and Cavara (see below), since isolations referred to the species are misdeterminations, they serve two purposes: (1) to indicate that aleuriospore measurements of random isolations of *S. chrysospermum* have much greater variation than is generally assigned to the species and hence would render very questionable any variety or species based upon this character, and (2) to show the similarity of these isolations to *S. chrysospermum* of common usage. As already noted, the isolations in question under the name *S. macrosporum* are, in my opinion, not that or-

ganism. In the discussion accompanying the original description, Saccardo and Cavara (6) mentioned that it was found growing with *Verticillium agaricinum* Link, a statement open to serious question in view of our present knowledge of the conidial form of *S. chrysospermum*. It cannot be conclusively proven without examination of the type specimen, but it seems highly probable that the *Verticillium* observed was merely the conidial form of the *Sepedonium*. Furthermore, perusal of the literature indicates that any hyphomycete having verticillately branched conidiophores growing on a member of the Agaricales is called *V. agaricinum*, and until the use of this name is stabilized, the determination of an organism of this species must be accepted with reservation, especially when accompanied by aleuriospores of a *Sepedonium*. For these reasons, I feel that *S. macrosporum* Sacc. & Cav. should be regarded as nothing more than a slightly larger-spored form of *S. chrysospermum*, and the name should be considered a synonym of that species.

The isolations referred to above and received from the Centraal-bureau as *S. macrosporum* are completely different from *S. chrysospermum* in their conidial form although aleuriospores have been shown to differ very little. This difference in conidial form is great enough to warrant recognition as a distinct species although exact identification of specimens must rest upon an examination of conidia rather than aleuriospores. The name, *Sepedonium ampullosporum*, is proposed for this organism because of the unique morphology of the conidia.

***Sepedonium ampullosporum* sp. nov.**

Coloniae primum albæ denique aureæ; hyphae steriles sparsæ vel copiosæ; aleurospores globosæ vel subglobosæ, tuberculis compositis, (10-)13-18(-22)  $\mu$  in diametro; conidiophora ascendentia vel recta, ramis septatis, hyalinis; rami terminales (phialidae) modo candelabrorum ordinatae; conidia singillatim vel in serie ferentes; conidia ampulliformia, apicibus nodosis, continua, hyalina, 10-20  $\times$  5-7.5  $\mu$ .

Colony white at first becoming pale yellow to brilliant golden and finally Xanthine Orange to Mars Yellow (Ridgway) in age; sterile hyphae sparse to copious, hyaline, sometimes becoming golden and encrusted in age, septate, measuring 5-6  $\mu$  in diameter; aleuriospores globose to subglobose, rough-tuberculate, golden-yellow microscopically, measuring (10-)13-18(-22)  $\mu$  in diam-

eter; conidiophores erect or ascending, branched, septate, hyaline, terminal branches (phialides) arranged in candelabra-like fashion, tips slightly flared, conidia borne singly or in succession at their apices; conidia bottle-shaped with larger central diameter tapering to a knobbed apex, ovoid conidia also produced; conidia 1-celled, hyaline, measuring 10-20  $\times$  5-7.5  $\mu$ .

This species has been found on members of the Boletaceae and Agaricaceae in North America, Europe, and the South American tropics. It probably has a worldwide distribution. Because of the general availability of the cultures, the one deposited in the Centraalbureau voor Schimmelcultures under the name *Sepedonium macrosporum* is designated the type culture.

Specimens examined: *Living material*: (1) isolation from *Boletus bicolor* Pk., S. C. Damon, July 1947, Kingston, R. I.; (2) isolation from *B. bicolor* Pk., coll. by W. H. Snell, Sept. 1947, near Highlands, N. C.; (3) isolation from *Strobilomyces floccopus* (Vahl. ex Fr.) Karsten, coll. by W. H. Snell, Aug. 1948, R. I. near Conn. line; (4) culture received from Centraalbureau voor Schimmelcultures, Baarn, Jan. 1951 (TYPE). *Herbarium material*: (1) Brazil, São Paulo, Viegas, 1944 (No. 4460), on *Hebeloma* sp.; (2) U. S. (?); No. 1364, no collection data, marked "unidentified" (Farlow Herbarium).

Still another conidial form, different from the two considered above, having aleuriospores referable to *S. chrysospermum* has been found; but I have seen only a single isolation of it and feel that no more than a mention of it can be made at this time. The aleuriospores of this isolation are like those of *S. chrysospermum* and *S. macrosporum* in all respects; but the conidia are produced in succession at the tips of long, usually unbranched, conidiophores. The conidia thus produced remain conglutinated together at the tip and are often surrounded by a drop of liquid. This type is referred to as the *Cephalosporium*-type and probably deserves a name in keeping with the principle followed above, but I do not feel that any definite proposal can be made until more isolations have been studied.

The second species to be given detailed consideration was isolated from *Gyroporus castaneus* (Bull. ex Fr.) Quélet and later from *B. edulis* Bull. ex Fries. It has also been observed on several

other occasions on the latter bolete, and appears to be rather common, though somewhat inconspicuous. Unlike the previously described (and most other) species of *Sepedonium*, this one does not have globose or subglobose aleuriospores. They are truncate-ellipsoid or truncate-obovate but never globose or subglobose at maturity, and are borne on short branches of the assimilative hyphae. This fungus also produces conidia of the *Verticillium*-type (FIG. 1, E). The colony is greyish-white to dirty cream color and may be very difficult to detect in the fresh condition. As the colony ages and the whole surface of the pileus and the mouths of the tubes are covered by the mycelium, it becomes more noticeable and appears as a white tomentum at maturity. Even when completely mature, the colony does not assume the color of the aleuriospores as is the case in *S. chrysospermum* but remains whitish until relatively late, when color finally becomes noticeable. Examination of fresh material shows very little except aleuriospores, and when conidia are observed it cannot be determined whether they are produced on the same mycelium as the aleuriospores or not.

In culture, the colony is slow-growing, requiring 7-10 days to cover an agar slant. It is usually white at first and growth is always closely appressed to the substratum. In only one isolation studied was there any distinct aerial mycelium. The colony grows in an asteroid fashion and after two weeks a pale yellow color appears in the center of the colony near the point of inoculation and spreads slowly and irregularly over the colony. The color of the mature colony varies from whitish cream to Naples Yellow or Warm Buff (Ridgway); and, as in other species, the color marks the maturation of the aleuriospores. The conidia are more abundant while the colony is still white (i.e., young), usually disappearing with the maturation of the aleuriospores. They are produced on erect or ascending, verticillately branched conidiophores and are abstricted from the tips of the branches, where they may be observed floating in a drop of liquid. The aleuriospores are produced singly and terminally on short hyphal branches in the typical manner of aleuriospores. They are smooth at first and somewhat subglobose, but soon become obovate to ellipsoid with a truncate base.

At maturity, they are golden yellow microscopically and have a much wrinkled surface (FIG. 1, F). Howell (2), studying the ontogeny of ornamentation in two species of *Sepedonium*, suggested that because the aleuriospores of *S. xylogenum* were only roughened, and the ornamentation was not formed as distinct outgrowths in the manner observed in *S. chrysospermum*, the species should be removed from *Sepedonium*. The application of the same reasoning to the species under present consideration is rejected because of the similarity in habitat and general appearance between this species and *S. chrysospermum*; and I agree with the Tulasnes (8) that it should be kept in *Sepedonium*. Furthermore, the heterogeneous nature of *Sepedonium* as it is now constituted makes it difficult to accept Howell's point of view.

The Tulasnes (8) described this fungus from members of the Boletaceae, especially *B. cyanescens* and *B. subtomentosus*, and placed it among the species of *Hypomyces* on the basis of its general appearance although they were not able to find an ascigerous stage. Later (9), they discussed this species and presented excellent illustrations of it. Saccardo and Fautrey (7) described the young stage of this fungus on a species of *Russula* as a new species under a new generic name, *Lejosepium aurcum* Saccardo & Fautrey. This fungus has also been distributed by Saccardo in the *Mycotheca Italica* under the name, *Sepedonium Tulasneanum* Saccardo. *Sepedonium Tulasneanum* was described by Saccardo from the aleuriospores of *Hypomyces Tulasneanus* as illustrated by Plowright (5). These aleuriospores were examined by the Tulasnes (9) under the name *H. luteo-virens*, and both they and Plowright illustrated them as spiny or tuberculate. Petch (4), in a re-examination of *H. luteo-virens*, described these same aleuriospores as verrucose, i.e., ornamented in the same manner as the aleuriospores of the fungus under discussion. The spores observed by Petch measure 18–27  $\times$  10  $\mu$ , slightly smaller than the spores of my fungus, but similar to it in other respects. Because of the smaller spore size given for *S. Tulasneanum*; and because the Tulasnes, who studied material of both species, regarded them as distinct species, I am maintaining them separate for the present. The specimens in the *Mycotheca Italica* have large spores and

cannot possibly be referred to *S. Tulasneanum* as that species is interpreted here.

**Sepedonium chlorinum** (Tulasne) comb. nov.

*Hypomyces chlorinus* Tulasne, Ann. Sci. Nat. Bot. IV, 13: 13. 1860.

*Lejosepium aureum* Sacc. & Fauntrey, Bull. Soc. Myc. Fr. 16: 24. 1900. ill.

Sterile hyphae present, usually within the substratum or closely appressed, hyaline, septate, becoming filled with colored material in age or disappearing, measuring 2-6  $\mu$  in diameter; conidiophores erect or ascending, hyaline, septate, verticillately branched, same diameter as sterile hyphae; conidia variable in size and shape, larger conidia tending to be ovate or subcylindrical, one-celled, hyaline, abstracted from ends of branches of conidiophore, conglutinated in drops of liquid, measuring 2-15  $\times$  1-6  $\mu$ ; aleuriospores large, produced on short hyphal branches, truncate-ellipsoid to truncate-obovate, golden in color microscopically, outer wall wrinkled longitudinally, measuring 26-42  $\times$  9-16  $\mu$ .

Found growing on members of the Boletaceae and Agaricaceae. Species upon which it is known to occur are *Bulgaria* sp., *Russula* sp., *Boletus cyanescens*, *B. subtomentosus*, *B. edulis*, *Strobilomyces floccopus*, and *Gyroporus castaneus*.

Type locality: Paris, France.

Specimens examined: *Living material*: (1) isolation from *Gyroporus castaneus* (Bull. ex Fr.) Quél., S. C. Damon, Oct. 1947, Kingston, R. I.; (2) isolation from *S. floccopus* (Vahl. ex Fr.) Karsten, S. C. Damon, Aug. 1948, Greenville, R. I.; (3) isolation from *Boletus edulis* Bull. ex Fr., S. C. Damon, Aug. 1950, Iowa City, Iowa. *Herbarium material*: (1) Italy; Massalonga, Verona, 1905, No. 1730, *Mycotheca Italica* (identified as *S. Tulasneanum* (New York Bot. Gard. Herb.)); (2) U. S.; Snell, Greenville, R. I., 1947 (Brown University Herb.).

ACKNOWLEDGMENTS

The species discussed in this paper constitute a part of a thesis submitted to the Faculty of Brown University in partial fulfillment of the requirements for the degree of Master of Arts, and are a part

of work continued at the State University of Iowa. I wish to express my gratitude to Dr. Walter H. Snell for his continued interest and encouragement throughout the course of the work, and for kindly making available the facilities of his department at times other than when I was connected with it. I also wish to thank Miss Joan Cox for help in making the drawings.

DEPARTMENT OF BOTANY,  
STATE UNIVERSITY OF IOWA,  
IOWA CITY, IOWA

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## A NEW SPECIES OF PHYSODERMA

M. S. BREWSTER<sup>1</sup>

(WITH 1 FIGURE)

Specimens of *Marsilea vestita* Hook. and Grev. growing in the greenhouse were observed in February of 1949 to be attacked by a fungus. These specimens had been collected the previous spring in a flooded area six miles north of Salina, Kansas, by P. H. Humboldt. Presumably they were diseased when brought into the greenhouse, but the area in question has not been revisited to observe the disease under field conditions.

The fungus causes golden-brown ellipsoid galls on petioles and leaflets. No galls have been seen on the rhizomes.

The petiole of *Marsilea vestita* has (like the rhizome) an amphiphloic, siphonostelic vascular cylinder with exarch protoxylem masses (FIG. A). In the rhizome the siphonosteles are externally and internally limited by an endodermal layer, but in the petiole the inner endodermis is often lacking or greatly reduced. Within the outer endodermis is a pericycle of a single row of cells. In submerged plants the center of the petiole is occupied by a pseudoparenchymatous pith. In plants growing on mud the pith may be more or less sclerotic; external to this is a ring of aerenchymatous tissue, surrounded by an outer layer of close parenchyma and then by the epidermis.

As a result of two dichotomies arising in close succession four leaflets are formed at the apex of the petiole. The anatomy of a leaflet resembles that of a dicotyledonous leaf, with netted venation and one full row and one half row of palisade cells.

The presence of the fungus results in ellipsoid galls restricted to the aerenchymatous tissue of the cortical regions of the petiole (FIG. 1A) and to the mesophyll in the leaflet (FIG. 1B). The expanded portion of the gall on the leaflet always occurred in a

<sup>1</sup> The author wishes to express her gratitude to Professor F. K. Sparrow, Jr., for his help in the identification of this fungus.

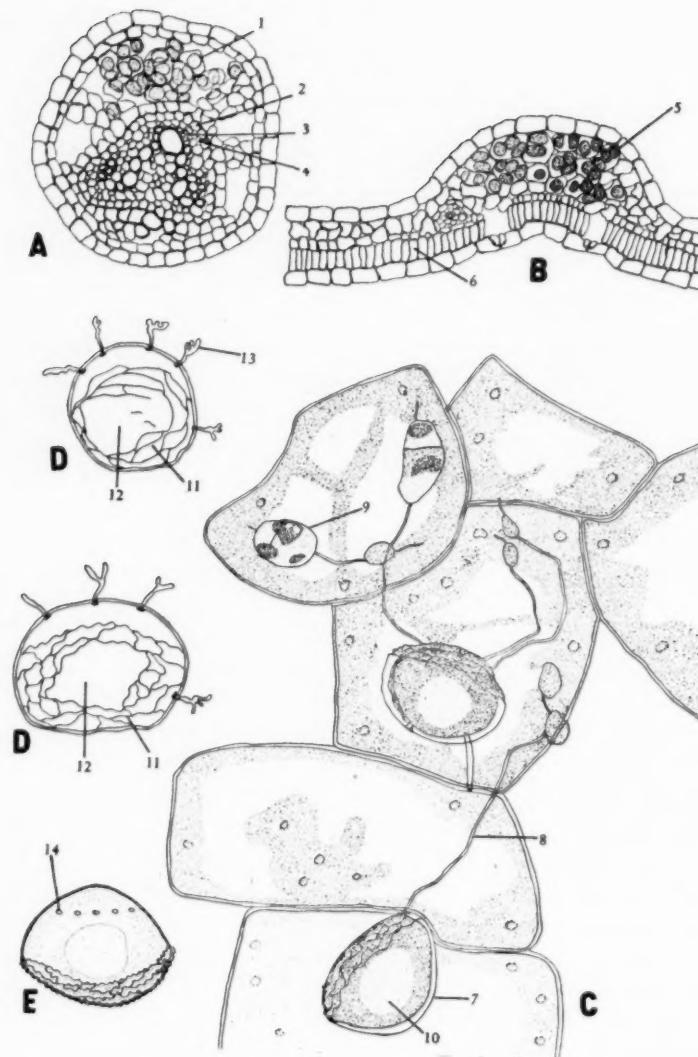


FIG. 1. A. Petiole showing very young gall histology and normal tissue. Resting spores (1), endodermis (2), protoxylem (3), pericycle (4),  $\times 100$ . B. Leaflet showing gall histology and normal tissue and expanded portion of leaflet and row of resting spores (5), and one-half of palisade cells (6),  $\times 100$ . C. Mature resting spore (7) within host cells, showing rhizoidal system (8) and turbinate organs (9). Also large central body in resting spore (10),  $\times 1790$ . D. Resting spore showing wrinkled area (11) and concave area (12) with antler-like haustorial processes (13),  $\times 1790$ . E. Mature resting spore showing wrinkled area and superequatorial ring of pits (14),  $\times 1790$ .

direction away from the row and a half of palisade cells. In neither the petiole nor the leaflet is the epidermis or the vascular tissue infected.

Within the infected area nearly every host cell contains from one to five (usually 2-4) resting spores of the fungus. The resting spores are pale brown, thick-walled, flattened on one surface, and connected to truncate turbinate organs by a very delicate rhizoidal system, features characteristic of the endobiotic phase of species of *Physoderma* (FIG. 1C). At the perimeter of the flattened area of the resting spore the outer wall is strongly wrinkled (FIG. 1D). During their development a ring of antler-like haustorial processes appears on each spore at approximately one-third of the distance above the equator. On the matured resting spore these processes evidently disintegrate, leaving only a ring of minute pits in the spore wall through which the haustoria are connected with the cytoplasm (FIG. 1E).

Sparrow (2) states that on morphological grounds there seems no justification for maintaining *Urophlyctis* apart from *Physoderma*. The only constant difference between the two genera is in their effect on the host. *Urophlyctis* induces strong gall formation while *Physoderma* may discolor and eventually kill the infected tissue without causing marked hypertrophy. In view of the foregoing, the fungus in question is placed in the type genus of the family, *Physoderma*, and is named *Physoderma marsileae* with the following description:

***Physoderma marsiliae* sp. nov.**

Thalli epibiotici monocentrici et sporangia ephemeralia nondum cognita. Thallus endobioticus extensus, polycentricus; rhizoidei fragiles ferentes organa 1-2-cellularia, truncata, turbinata plerumque longa  $15.2-14.6 \mu \times 9.3-8.4 \mu$  diam. et sporangia resistentia membranis crassis; in folio et petiolo pustulas claras erectas fuscas ellipsoidales usque ad 2 mm. diam. formantia; sporangiis resistentibus maturis aggregatis, supra orbiculatis, infra subconcavis, lateribus processus multos breves ramosos corniformes supra-equitoriales ferentibus; membrano exteriore includente tenui, sporangii tertia parte inferiore rugosa; longa  $32.9-18.9 \mu \times 23.0-17.5 \mu$  lata, plerumque  $25.8 \times 22.1-18.1 \mu$ ; intus subgranularia plerumque globulum magnum centrale includentia; membrana succinea  $2.5 \mu$  crassa. Germinatione, ut videtur, dehiscentia circumscissili centralis partis superficie concavae.

Epibiotic monocentric thalli and ephemeral sporangia at present unknown. Endobiotic thallus extensive, polycentric, its delicate rhizoids bearing 1-2-celled truncate turbinate organs which average  $15.2-14.6 \mu$  long  $\times 9.3-8.4 \mu$  in diameter, and thick-walled resting spores; forming on leaf and petiole distinct, raised, dull brown ellipsoid galls up to 2.0 mm. in greatest diameter. Resting spores at maturity aggregated, circular in outline from above with lower surface slightly concave in side view, bearing several short-branched, antler-like supra-equatorial processes; the ensheathing thin outer wall strongly wrinkled over the lower one-third of the spore;  $32.9-18.9 \mu$  long  $\times 23.0-17.5 \mu$  wide, the majority being  $25.8 \times 22.1-18.1 \mu$ ; content slightly granular and usually containing a large central globule; wall amber-colored,  $2.5 \mu$  thick. Germination apparently by the circumscissile dehiscence of the central portion of the concave area.

Type specimen deposited in the Mycological Herbarium at the University of Kansas, Lawrence, Kansas.

Species of *Physoderma* are reported as parasites on many phanerogamic hosts but until now are not known to attack any of the Pteridophytes. Sparrow (1943) reports two genera of Chytrids, *Rhizophydioides* and *Rhizophylyctis*, on spores of certain Pteridophytes. There is but one fern, *Aspidium* sp., in this list.

Apparently the only previously known fungus parasite of *Marsilea* is *Phoma Marsiliae*, reported by Tassi (1) in 1899 as occurring in the petiole of *Marsilea quadrifoliata* L. in Europe (Oudemans, 1919).

DEPARTMENT OF BOTANY,  
UNIVERSITY OF KANSAS,  
LAWRENCE, KANSAS

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# HOMOTHALLISM VS. HETEROOTHALLISM IN THE *PENICILLIUM LUTEUM* SERIES<sup>1</sup>

KENNETH B. RAPER AND DOROTHY I. FENNELL

(WITH 1 FIGURE)

## INTRODUCTION

*Penicillium luteum* was described by Zukal in 1889. Four years later the species formed the subject of a paper by Wehmer (1893). In both of these publications, cultural characteristics and details of morphology were discussed at considerable length. Both papers were well illustrated, and both investigators emphasized the unique ascospores of this species—ascospores that are characterized by three or four transverse bands (FIGS. 1, A, B). Neither Zukal nor Wehmer worked with strains derived from single spores, and neither seemed to have experienced difficulty in obtaining perithecia, asci and ascospores. It is surprising that a species so well described and so thoroughly reported received little attention by mycologists during the three ensuing decades.

In 1925 and 1926, H. G. Derx published two short papers in the Bull. Soc. Mycol. de France and the Trans. Brit. Mycol. Soc., respectively, in which he presented strong evidence of heterothallism in an isolate designated, "*Penicillium luteum* (Zukal?) Wehmer certissime!" The two papers were marked as preliminary notes. They are almost identical in content, straightforward in presentation, and represent models of succinctness. The evidence, as reported, is convincing and conclusive. Kniep fully accepted these papers, and included in his book, "Die Sexualität der Niederen Pflanzen" (1928), Derx's table of data together with a schematic diagram of the life cycle of *P. luteum* based upon this work. Shear and Dodge, in their paper on heterothallism in *Neurospora* (1927), stated that Derx had "proved conclusively that *Peni-*

<sup>1</sup> Report of a study in which certain phases were carried out under the Research and Marketing Act of 1946.

*cillium luteum* is heterothallic" (see page 105). Other investigators, including Lindegren and Andrews (1945), have accepted Derx's work, apparently without reservation.

Why then should there be any question regarding heterothallism in *Penicillium luteum*?

The question stems from the fact that in the laboratory subsequent investigators, including the writers, have been unable to substantiate Derx's claims. In his careful investigation of ascocarp formation in species of *Penicillium*, Emmons (1935) failed to find any evidence of heterothallism. His study included 10 ascosporic species, of which 6 belonged to the *Penicillium luteum* series, and 1 represented the species *P. luteum* Zukal in what is believed to constitute its typical form (FIG. 1, C). Members of the *P. luteum* series investigated by Emmons included *P. wortmanni* Klöcker, *P. spiculispernum* Lehman, *P. bacillusporum* Swift, *P. vermiculatum* Dangeard, *P. stipitatum* Thom, and *P. luteum* Zukal. The four additional species studied, not closely related to *P. luteum*, were *P. javanicum* van Beyma, *P. ehrlichii* Klebahn, *P. egyptiacum* van Beyma and *P. brefeldianum* Dodge.

#### EXPERIMENTAL

During investigations leading to the preparation of the "Manual of the Penicillia" (Raper and Thom, 1949), scores of ascosporic Penicillia were examined, including several new species. This latter group included three new members of the *P. luteum* series, described by Raper and Fennell (1948), namely: *P. helicum*, *P. rotundum* and *P. striatum*. In addition, some ascosporic strains of *P. avellanicum* Thom and Turesson were encountered. Thus, we had available for study four ascosporic species which were unavailable to Emmons. Possessing this additional material, and with the encouragement of Drs. B. O. Dodge and Charles Thom, we have endeavored to obtain further information regarding the question of heterothallism in *P. luteum* Zukal, and in the series of species more or less closely related to it. Fortunately for this study, we received recently from Dr. J. H. Warcup, Cambridge University, England, four new isolates of *P. luteum* Zukal, obtained by him from soil (NRRL 2235 and 2246) or from the roots of spruce seedlings (NRRL 2217 and 2248).

The present study has been divided roughly into two parts. In 1949, preliminary investigation of 8 species was undertaken, at which time 10 or 12 single ascospores of each were isolated (TABLE I); and the colonies developing therefrom were subsequently examined for the presence of ascocarps, asci and ascospores. The results of these tests are summarized in TABLE I. Single spore isolations were made by the method described by Thom and Raper (1945, pp. 42-44), and in all cases spores selected for isolation

TABLE I  
ASCOSPORE PRODUCTION BY MONO-ASCOSPORE ISOLATES IN MEMBERS  
OF THE *Penicillium luteum* SERIES

Species	Strain	First trial—1949			Second trial—1950		
		Spores isolated	Colonies produced	Ascospores produced	Spores isolated	Colonies produced	Ascospores produced
	NRRL						
<i>P. luteum</i> Zukal	2102	12	12	12	20	20	20
<i>P. luteum</i> Zukal	2235				29	27	27
<i>P. luteum</i> Zukal	2246				21	21	21
<i>P. luteum</i> Zukal	2247				24	24	24
<i>P. luteum</i> Zukal	2248				24	24	24
<i>P. luteum</i> Zukal	2103*				20	19	19
<i>P. avellaneum</i> Thom and Turesson	1938	10	9	9	20	16	16
<i>P. bacillusporum</i> Swift	1025				20	20	20
<i>P. helicum</i> Raper and Fennell	2106	10	9	9	20	20	20
<i>P. rotundum</i> Raper and Fennell	2107	10	7	7	20	20	20
<i>P. spiculiflorum</i> Lehman	1028				20	20	20
<i>P. stipitatum</i> Thom	1006	10	10	10	20	19	19
<i>P. striatum</i> Raper and Fennell	717	10	9	9	20	20	20
<i>P. vermiculatum</i> Dangeard	2098	10	10	10	20	20	20
<i>P. wortmanni</i> Klöcker	1017	10	8	8	20	19	19

\* Not typical of *P. luteum* Zukal; strain produces broadly spreading colonies, but ascospores show spiral bands, hence it is included with that species.

were allowed to develop short germ tubes (usually within 1-3 days) before being removed to fresh agar plates for colony development. Spores were germinated upon malt extract agar, or upon Czapek solution agar enriched with 1% corn steep liquor, and were subsequently transferred to malt agar. As shown in TABLE I, a limited number of these spores failed to develop colonies. However, in every case where a colony developed, that culture was characterized by normal ascocarps, abundant asci, and ascospores entirely characteristic of the species.

We repeated this study during 1950. Twenty or more single germinating ascospores were isolated from each of the 8 species

previously examined, and from 2 additional species, *P. bacillosporum* and *P. spiculisorum*. We included also the four Warcup isolates of *P. luteum* and a strain, NRRL 2103 from Swedish soil, which is regarded tentatively as an atypical representative of this species. This latter culture is characterized by broadly spreading colonies, unlike typical strains of *P. luteum*, but marked by the production of ascospores with definite though inconspicuous transverse ridges. In this study, as in the previous one, occasional germinated ascospores failed to develop colonies when transplanted on fresh substrata. *In every case, however, the colonies which developed were characterized by typical and abundant ascocarps, asci and ascospores.*

Our results are thus in complete agreement with those reported by Emmons (1935). Neither in *Penicillium luteum* nor in any related species have we obtained any evidence of heterothallism. Any reproductive cell, ascospore or asexual conidium, is capable of yielding a colony in which the complete cycle of the fungus is reproduced. Single conidia invariably and promptly developed heavily ascosporic colonies indistinguishable from those produced by single ascospores, except in *P. luteum* NRRL 2102. In this strain some conidia immediately developed heavily ascosporic colonies, whereas others produced colonies that were predominantly conidial. Mixing the latter in all possible combinations failed to hasten or increase ascospore production. Some factor associated with nutrient or water depletion seemed to be responsible for initiating the ascigerous phase in these clones. Each, however, retained the capacity to produce ascospores under proper but as yet undefined conditions.

#### DISCUSSION

How, then, shall the work and published papers of Derx be interpreted? A possible explanation is that he was working with a unique strain in which the sexes, or mating types, were segregated during ascospore formation. An alternative explanation might be, as Emmons suggested (1935, p. 143), that Derx encountered "some type of variation" which occurred in a pattern that simulated heterothallism. What is the evidence?

Derx's papers must stand on their own merit. The collaborative support suggested by the publications of Kniep (1928), Shear and Dodge (1927), and Lindegren and Andrews (1945) is, in fact, no support at all. Kniep, as a monographer, merely included in his treatise a summary of what appears to be an exquisite demonstration of heterothallism in a group of fungi for which no similar claims had been made previously. Shear and Dodge (1927) likewise cited the literature wherein another fungus, *P. luteum*, was reported to behave in a manner comparable to that which they were then demonstrating for *Neurospora crassa* n. sp. and *Neurospora sitophila* n. sp. Neither of these authors undertook studies of the developmental cycles of ascosporic Penicillia until somewhat later (Dodge, 1933; Shear, 1934). Lindegren and Andrews (1945) cite the statement of Shear and Dodge (1927), but give no indication of having carefully investigated single spore isolates of *P. luteum*.

Neither Emmons (1935) nor the present investigators have found the slightest evidence of heterothallism in any strain of *Penicillium*. Seventeen strains, representing 6 species, were included in the former study, whereas 15 strains representing 10 species, and including 5 isolates of *Penicillium luteum* Zukal, have been carefully examined in the present work. In addition to this number, scores of other isolates of ascosporic Penicillia, world-wide in origin, have been handled in this Laboratory over a period of many years without encountering evidence of heterothallism. The majority of these latter cultures represented strains freshly isolated from soil and other natural sources.

We have observed that some ascosporic Penicillia during long periods of laboratory cultivation lose their capacity to develop fertile ascocarps unless they are carefully transferred and maintained upon suitable culture media. Emmons (1935), Thom (1930), Raper and Thom (1949) and others have called attention to this phenomenon. The tendency of cultures to become sterile is particularly marked in some species, such as *P. avellanicum*, and is less frequently encountered in others, such as *P. wortmanni*, *P. vermiculatum*, and *P. spiculisorum*. The basic causes which underlie the loss of the ascosporic stage are not understood and

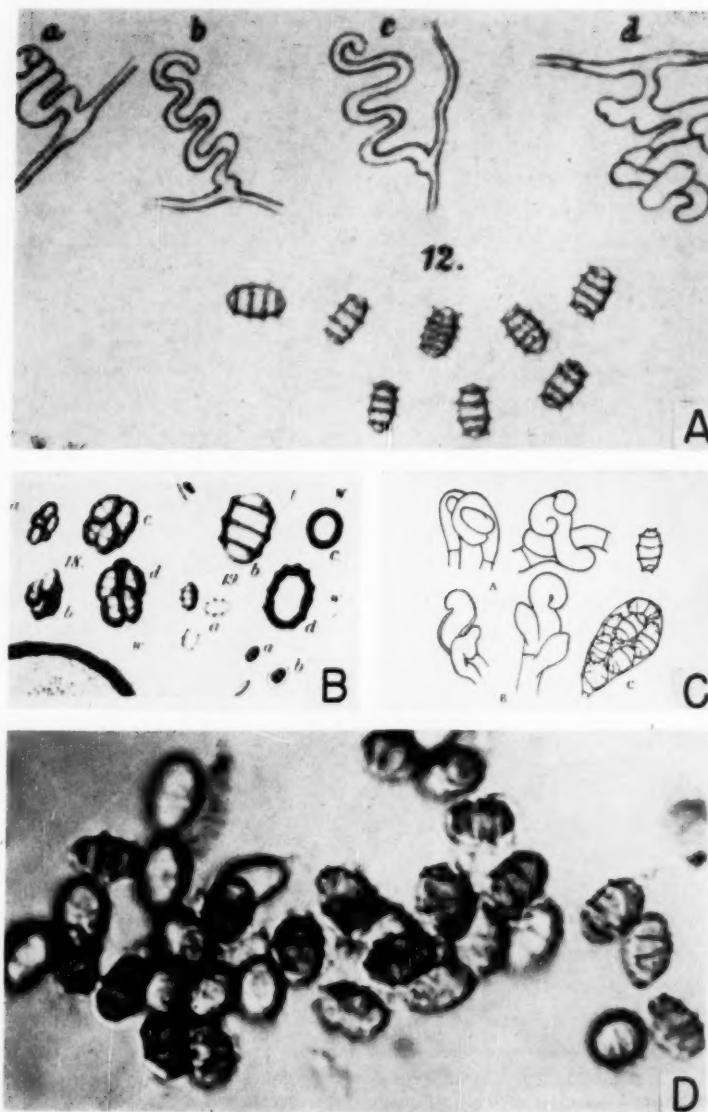


FIG. 1. Ascospores of *Penicillium luteum* Zukal as illustrated by Zukal (A), Wehmer (B), and Emmons (C), and as observed by the writers (D). *A*, Fig. 12 and a portion of Fig. 5 (initial stages in ascocarp formation) of Zukal's Taf. III,  $\times 1800$ ; *B*, Figs. 18 and 19 of Wehmer's Taf. XXV, scale

merit careful study. Whatever the reason for their origin, no case is known to the writers where such a non-ascosporic culture of *Penicillium* has ever regained its capacity to produce perithecia and ascospores. The uniqueness of a strain which behaved in the manner reported by Derx is thus further emphasized.

Questions may arise concerning the correct identification of Derx's culture as representing *P. luteum* Zukal. Of this there can be little doubt. He had before him four ascosporic strains, representing *P. wortmanni* Klöcker, *P. vermiculatum* Dangeard, *P. luteum* Zukal, and an unidentified form from CBS which had come originally from Dr. A. Richards as *P. luteum*. Their differences were obviously clearly understood, and, it may be added, his discussion reveals an appreciation of Dangeard's species beyond that of other students of *Penicillium* at that time. His brief description and notes leave no doubt of his familiarity with Zukal's and Wehmer's concept of *P. luteum* as revealed in their publications. There is every reason to believe, as he states, that he was dealing with a fungus which fulfilled Wehmer's specifications for *P. luteum*, and differed from Zukal's description principally in producing ascospores with transverse ridges smooth rather than verruculose.

Subcultures of *Penicillium luteum* NRRL 2102 and NRRL 2103 (TABLE I) were forwarded to Professor Derx at the Treub Laboratorium, Buitenzorg, Java, in August 1950. He has subsequently informed us that neither of these strains conforms with that which he investigated, and notes that "more stress should have been laid on the purpurogenous qualities of this [his] mold." Derx may thus have worked with some strain possessing unusual and possibly unique cultural characteristics, although under certain conditions the production of red to purplish pigments is a common and variable characteristic of the Biverticellata-Symmetrica group of *Penicillia* generally. The pattern of the ascospores in *P. luteum* Zukal (FIG. 1, A) is, in any case, sufficiently distinctive to preclude the possibility that Derx (1925, 1926), Emmons (1935), or the writers may have incorrectly identified this species among the cultures with which they worked. Still, had this occurred, the

variable, but *b* and *d* of latter, *ca.*  $\times 2000$ ; C, Fig. 10 of Emmons, showing ascospore, young ascus, mature ascus, and ascocarp initials,  $\times 1050$ ; D, photomicrograph of ascospores in NRRL 2102 (see Table I),  $\times 2000$ .

picture would not be altered, for neither Emmons nor the writers have found any evidence of heterothallism in *any* ascosporic *Penicillium* investigated.

By dilution plating and subsequent isolation, Derx secured a number of subcultures which differed in physiological characteristics (e.g., gelatin liquefaction and color production) and in conidial pigmentation, some of these producing dark olive-green conidia whereas others produced bluish gray-green conidia. None of these isolates produced fertile perithecia. On the other hand, such perithecia were often produced after some weeks in the original dilution plates, which presumably contained many contiguous colonies.

Derx's attention was next directed to the isolation of strains from single ascospores. Here his experimental results are clearly and beautifully set forth, and if any criticism of this work can be made it is that the data are too consistent, too symmetrical. Twelve ascospores were isolated,<sup>2</sup> of which 6 represented one sex (-) and 6 another (+). Of both (-) and (+) strains, whose symbols were arbitrarily assigned, there were 2 which showed "the greatest sexual polarity." In each of these 4 strains "sterile haploid ascocarps" were produced, and the greatest abundance of fertile perithecia developed when from among these, strains of opposite sex were paired (TABLE II, and Derx 1925 and 1926).

Insofar as is known, Derx never distributed cultures of his fungus. In checking back through correspondence of that date with Thom, it is found that cultures were promised but never received. Thom, however, did send Derx a group of ascosporic *Penicillia*, which are mentioned in his papers as material around which he hoped to develop a subsequent communication. This more extensive publication never materialized, and so we are left with the two, all too brief, preliminary notes. Derx's cultures were apparently lost soon afterward when he returned to industrial work.

Derx's work has not been repeated, perhaps for lack of the particular strain of *Penicillium luteum* with which he worked. For the same reason it cannot be convincingly disputed. His mycological studies were carried out in Professor Kluyver's Laboratory

<sup>2</sup> The impression gained from Derx's papers that only 12 mono-ascospore cultures were isolated has been confirmed through recent correspondence.

of Microbiology in the Technical University at Delft, and he acknowledged the indispensable aid of C. B. van Niel (then Curator of the same Laboratory) in the isolation of single ascospores using the apparatus of Janse and Peterfi. Favored by the guidance and counsel of these men, it is difficult to comprehend how serious errors might have been made.

Two recently published papers may point to a possible explanation. Leupold (1950), working with a yeast identified as *Schizosaccharomyces Pombe* Linder, str. *liquefaciens* (Osterwalder), found that starting with a homothallic single spore culture, heterothallic strains of both mating types could be obtained from it after

TABLE II

Mono- ascospore strain	A	B	C	D	E	F	G	H	I	K	L	M
A(-)	—											
B(+)	1	—										
C(+)	1	—	—									
D(-)	—	2	2	—								
E(-)	—	3	3	—	—							
F(-)	—	3	3	—	—	—						
G(-)	—	1	1	—	—	—	—					
H(+)	2	—	—	3	4	4	2	—				
I(+)	1	—	—	2	3	3	1	—	—			
K(-)	—	1	1	—	—	—	—	2	1	—		
L(+)	2	—	—	3	4	4	2	—	—	2	—	
M(+)	1	—	—	2	3	3	1	—	—	1	—	—

The numbers indicate relative quantity of perithecia developed.

— = none; 1 = very feeble; 2 = feeble; 3 = good; 4 = abundant.

Strains E, F, H, and L show greatest sexual polarity and produce sterile ascocarps.

Table II. Adapted from Table I of Dux's paper in Bull. Soc. Myc. France 41: 381, 1925.

six months' cultivation. Conversely, starting with heterothallic strains of either (+) or (-) mating types, richly sporulating homothallic cultures could be obtained as spontaneous sector mutations in hanging drop preparations. In the same year, Olson succeeded in isolating heterothallic strains of *Ceratostomella fimbriata* (Ell. & Hals.) Elliott. When the former were crossed, the progeny included isolates apparently identical with the original homothallic strain. Long before this, Dodge (1927) and Shear and Dodge (1927) had shown that their species *Neurospora tetrasperma*, which is typically homothallic and normally produces asci contain-

ing four binucleate-bisexual ascospores, might upon occasion develop 5-spored ascii wherein two spores were small, uninucleate and unisexual—hence gave rise to heterothallic races. The situations are not strictly comparable, but each case emphasizes the fact that a particular species and strain of ascomycete is capable of existing in either a homo- or heterothallic state. It would seem equally possible that *P. luteum* Zukal might show a similar duality. The same in turn might be expected of other members of the *P. luteum* series, all of which are ascosporic (and according to our tests, homothallic). However, if such duality exists, is it not strange that neither Emmons nor the writers have encountered any evidence of heterothallism in any of the 32 strains studied intensively by them, or among the scores of other isolates belonging to this series which have been handled and observed in this laboratory without detailed mono-ascospore analysis?

If sufficient ascosporic members of the *Penicillium luteum* series from enough different areas are examined, perchance we shall find a strain which behaves in the manner reported by Derx. However, as matters now stand, if we accept Derx's work without reservation we are forced to conclude that he was singularly fortunate in his discovery of a strain which, up to this time, appears unique, not only for the species *Penicillium luteum*, with which he worked, but also for the much larger series of ascosporic Penicillia to which it belongs.

The aid of other mycologists is solicited in our attempts to resolve this paradox.

#### ACKNOWLEDGMENT

The writers are indebted to Dr. Charles Thom for making available copies of his correspondence with Dr. Derx during the period of the latter's work on *Penicillium luteum*.

#### SUMMARY

Sixteen members of the *Penicillium luteum* series, representing 10 species and including 5 typical strains of *P. luteum* Zukal, have been analyzed by means of mono-ascospore isolates. All were found to be homothallic. Other species under investigation included *P. avellaneum* Thom and Turesson, *P. bacillosporum* Swift, *P. helicum*, *P. rotundum*, and *P. striatum* Raper and Fennell, *P.*

*spiculiflorum* Lehman, *P. stipitatum* Thom, *P. vermiculatum* Dangeard and *P. wortmanni* Klöcker. The singular nature of Derx's results, wherein *P. luteum* was reported to be heterothallic (1925 and 1926), is discussed.

FERMENTATION DIVISION,  
NORTHERN REGIONAL RESEARCH LABORATORY,  
PEORIA, ILLINOIS

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## NOTES ON FLORIDA AGARICS

W. A. MURRILL

### **Cortinarius Birchfieldii** sp. nov.

Pileo convexo-subexpanso, 6 cm. lato, glabro, violaceo-albo, subgrato; lamellis adnatis, 6 mm. latis; sporis obovoideis, levibus,  $10 \times 5 \mu$ ; stipite concolori, glabro,  $4 \times 2.5$  cm., basi emarginato-bulboso, 3.5 cm. lato; cortina infera, copiosa.

Pileus convex to subexpanded, solitary, 6 cm. broad; surface dry, smooth, glabrous, uniformly satiny-white with a violet tint, margin entire to somewhat scalloped; context white to slightly discolored, up to 1 cm. thick, odorless, mild, becoming slightly bitter; lamellae adnate with decurrent edges, moderately close, inserted, 6 mm. broad, entire, pallid; spores obovoid, smooth, inequilateral, bright-ferruginous, about  $10 \times 5 \mu$ ; cystidia none; stipe solid or hollow, smooth, glabrous, concolorous, equal and  $3 \times 2.5$  cm. above the short, concolorous, distinctly margined bulb 3.5 cm. in width; cortina copious, basal and marginal.

Type collected by Wray Birchfield in leaf-mold under a laurel oak in Gainesville, Fla., Jan. 5, 1951 (F 40858). Found but once. A beautiful species.

### **Cortinarius laeticolor** sp. nov.

Pileo convexo-subexpanso, gregario, 6 cm. lato, viscido, badio, margine flavo, grato; lamellis citrinis, 7 mm. latis; sporis obovoideis,  $7 \times 4 \mu$ ; stipite solidio, subclavato, citrino, glabro,  $2.5 \times 1.8-2.2$  cm.; cortina infera, evanida.

Pileus convex, not fully expanding, gregarious, about 6 cm. broad; surface viscid, glabrous, bay, margin incurved, entire to lobed, flavous or paler yellow; context 6 mm. thick, pale-yellow, unchanging, mild, with musty odor; lamellae lemon-yellow, unchanging, medium distant, adnexed, rounded behind, entire, 7 mm. broad; spores obovoid, uniguttulate, bright-ferruginous, slightly roughened, about  $7 \times 4 \mu$ ; stipe solid, equal above the slightly enlarged base, dry, smooth, glabrous, pale-yellow, not margined, about  $2.5 \times 1.8-2.2$  cm.; cortina basal, scant, vanishing.

Type collected by W. A. Murrill on a grassy lawn under a laurel oak in Gainesville, Fla., Jan. 7, 1951 (F 41349). Not found elsewhere. Evidently a winter-fruiting species. The colors are varied and striking.

**Tricholoma russuloides** sp. nov.

Pileo convexo-subexpanso, 7-9 cm. lato, glabro, albo-cremeo-roseo, grato; lamellis confertis, adnexis, albis; sporis ovideis, levibus,  $8-9 \times 4.5 \mu$ ; cystidiis longis, albis; stipite albo, glabro,  $5 \times 1.5-2.5$  cm.

Pileus convex to subexpanded, solitary or gregarious, 7-9 cm. broad; surface slightly viscid when wet, smooth, glabrous, rosy with cream and white areas, margin entire, paler; context white, unchanging, firm, odorless, mild; lamellae adnexed, plane, medium broad, inserted and forked, crowded, white, becoming brown in the herbarium; spores amyloid, ovoid, smooth, hyaline,  $8-9 \times 4.5 \mu$ ; cystidia long, pointed, hyaline, projecting far beyond the basidia; stipe solid or stuffed, dry, smooth, glabrous, tapering downward, about  $5 \times 1.5-2.5$  cm.

Type collected by W. A. Murrill under live-oak in Gainesville, Fla., May 29, 1938 (F 16268). Common in the Gainesville region under evergreen oaks. A beautiful species, which in my nomenclature would be **Melanoleuca russuloides** (Murrill) comb. nov.

**TRICHOLOMA RUTILANS** (Schaeff.) Quél.

A fine collection of this beautiful species was made by Dr. G. F. Weber on April 15, 1950, in moist mixed woods a few miles northwest of Gainesville, Fla. The hymenophores were scattered along the side of a decaying log of loblolly pine lying near a small stream. All of them were covered with purplish tomentum; none were yellowish and streaked as in Bresadola's colored plate. The large cystidia, however, were exactly as figured by him, although the spores averaged somewhat smaller. Bresadola says the species is edible. Dr. Weber found some specimens nibbled by squirrels. Peck did not list it as edible, possibly because it was seldom found by him.

This species is widely distributed in Europe and North America, occurring on old stumps or logs of conifers in coniferous or mixed

woods. Kauffman found it on pine, balsam and hemlock. Bresadola reports it on coniferous trunks in Europe, North America, Japan and Australia. I have found it a few times about Gainesville on pine logs and stumps; also once near St. Augustine. Being a northern species, one would expect it to occur in cool weather rather than during our hot summer season. This is true; I made a good collection of it on April 12, 1938, at Magnesia Springs, several miles east of Gainesville. My name for the species is *Cortinellus rutilans* (Schaeff.) Karst.

UNIVERSITY OF FLORIDA,  
GAINESVILLE, FLORIDA

## THE SENSITIVITY OF BLASTOMYCES DERMATITIDIS TO ANTIFUNGAL AGENTS<sup>1</sup>

ELIZABETH W. ULRICH, LOYAL S. SUTER AND WOLCOTT B. DUNHAM

Little is known about the *in vitro* sensitivity of *Blastomyces dermatitidis* to agents which might prove efficacious in the treatment of blastomycosis, a not uncommon disease for which little can be done specifically. Littman, Wicker, and Warren (5) found that two strains of the organism were resistant to streptomycin and penicillin in concentrations up to 200 units per ml. Whiffen (7), employing a single strain, found that 1000 micrograms of actidione inhibited growth. Campbell, Saslaw, and Strong (1) reported that aureomycin, polymyxin B, chloromycetin, and neomycin exhibited a marked inhibitory effect against *B. dermatitidis*. Salle, Jann, and Ordanik (6) found that lupulon had no demonstrable effect on a number of molds and actinomyces, but *B. dermatitidis* was not included in the study. Landy, Warren, Roseman and Colio (4) found that growth of the mycelial form was restricted by 0.0005 mg. per ml. of bacillomycin and that the yeast phase gave no growth with 0.0025 mg. per ml. The present study was undertaken for the purpose of increasing the present knowledge of the sensitivity of *B. dermatitidis* to various agents *in vitro*.

### METHODS

In each case 6 strains of *B. dermatitidis* were tested concurrently. Four of these strains had been isolated from patients at this general hospital, and, in order to facilitate repetition of any part of this study, the other two were well known strains secured

<sup>1</sup> Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

from the American Type Culture Collection. Agents tested were as follows: ethyl vanillate (Sterling Winthrop Research Institute), potassium iodide (USP, granular, Merck), undecylenic acid (Decyl Pharmacal Company), actidione (Upjohn Company), bacillomycin B (Wyeth Institute of Applied Biochemistry), antibiotic X-G (Chas. Pfizer & Co. Inc.) (3), lupulon (Lot #H-7-123B, U. S. Dept. Agriculture, Western Regional Research Laboratory, Albany, Calif.), humulon (Phenylenediamine salt, U. S. Dept. Agriculture, Western Regional Research Laboratory, Albany, Calif.), aureomycin, and terramycin. Aqueous stock solutions were prepared of the following agents: potassium iodide, actidione, aureomycin, and terramycin. Ethyl alcohol stock solutions were prepared of the other agents. The alcoholic solutions were appropriately diluted using as a diluent the culture medium in the fluid state. The highest concentration of alcohol attained in any dilution was 2.0 per cent prior to heating. All the media containing alcohol were heated in an autoclave for 5 minutes at approximately 3 pounds pressure, after which they were allowed to solidify as slants. Control tubes of media prepared with 2 per cent ethyl alcohol and heat treated as above were capable of producing excellent growth. Kelley's blastomyces medium was used throughout, except that, in addition, aureomycin was tested on Sabouraud's medium, the latter being employed since aureomycin is more stable in an acid medium (2). Incubation was at 37° C., thereby employing the yeast phase of *B. dermatitidis*. Kelley's medium is prepared as follows: 10 grams peptone (Bacto), 10 grams dextrose, 5 grams sodium chloride, 3 grams beef extract and 20 grams agar are dissolved in 980 ml. distilled water at 100° C. and then cooled to 50° C., after which 20 ml. of laked blood (5 ml. of blood plus 15 ml. distilled water) are added. The medium is sterilized for 25 minutes at 15 pounds pressure.

#### RESULTS AND CONCLUSIONS

The results are given in TABLE I. These indicate that under the conditions of the experiment *B. dermatitidis* is resistant, *in vitro*, to terramycin, aureomycin, and potassium iodide. There is some

TABLE I  
DEGREE OF GROWTH OF *B. dermatitidis* IN THE PRESENCE OF VARIOUS AGENTS

Agent and Strain of <i>B. dermatitidis</i>	Milligrams of agent per milliliter						Agent and Strain of <i>B. dermatitidis</i>	Units of agent per milliliter				
	0	.0001	.001	.01	.1	1		0	.01	.1	1	10
Potassium Iodide							Actidione					
6573*	4***		4	4	4	4	6573	4	1	2	0	
8757*	4		4	4	4	4	8757	4	1	0	0	
7103*	3		3	3	3	3	7103	3	1	0	0	
8050*	4		4	4	4	4	8050	4	2	0	0	
7967**	4		1	4	4	4	7967	4	4	0	0	
10225**	4		4	4	4	4	10225	4	3	0	0	
Undecylenic Acid							Antibiotic X-G					
6573	4	4	4	4	0***		6573	4	4	2	0	
8757	4	4	1	0	0		8757	4	1	0	0	
7103	3	2	1	0	0		7103	3	2	0	0	
8050	4	4	4	1	0		8050	4	1	1	0	
7967	4	4	3	0	0		7967	4	4	0	0	
10225	4	4	2	1	0		10225	4	1	0	0	
Ethyl Vanillate							Bacillomycin B					
6573	4		4	4	0		6573	4	4	4	0	
8757	4		4	1	0		8757	4	1	1	0	
7103	3		2	1	1		7103	3	2	2	0	
8050	4		4	1	0		8050	4	1	1	0	
7967	4		4	4	0		7967	4	4	3	0	
10225	4		4	4	0		10225	4	2	1	0	
Lupulon							Terramycin					
6573	4		4	4	3	0	6573	4	4	4	4	
8757	4		4	1	0	0	8757	4	4	4	4	
7103	3		2	1	1	0	7103	3	4	4	4	
8050	4		4	3	1	0	8050	4	4	4	4	
7967	4		3	3	1	0	7967	4	4	4	4	
10225	4		4	4	0	0	10225	4	4	4	4	
Humulon							Aureomycin	0	3	6	12.5	25
6573	4		4	4	1	0	6573	4	2	2	2	
8757	4		4	4	0	0	8757	4	4	4	3	
7103	3		2	2	1	0	7103	3	3	3	3	
8050	4		4	4	1	0	8050	4	4	4	0	
7967	4		3	3	3	0	7967	4	4	4	4	
10225	4		4	3	1	0	10225	4	4	4	4	

\* Strains of *B. dermatitidis* isolated at Kennedy V. A. Hospital.

\*\* Strains of *B. dermatitidis* secured from American Type Culture Collection.

\*\*\* 4 represents heavy confluent growth; 0 represents no growth.

inhibition of growth with relatively high dilutions of lupulon, humulon, ethyl vanillate, actidione, and bacillomycin B. Of the agents tested the most striking inhibitory effects were obtained with undecylenic acid and antibiotic X-G.

GENERAL RESEARCH LABORATORY, VETERANS ADMINISTRATION

MEDICAL TEACHING GROUP,

KENNEDY HOSPITAL, MEMPHIS, TENN.

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## STROMATINIA NARCISSI, A NEW, SEXU- ALLY DIMORPHIC DISCOMYCETE<sup>1</sup>

F. L. DRAYTON<sup>2</sup> AND J. W. GROVES<sup>3</sup>

(WITH 8 FIGURES)

Sclerotia of three kinds are commonly found on the outer scales of narcissus bulbs. The largest of these, which vary from 1-2.5 mm. in diameter, are irregular in shape, closely adherent, and more or less globose, and are formed by the fungus responsible for the smoulder or grey mould disease attacking the leaves and flowers. This fungus has been known as *Botrytis narcissicola* Kleb. but Gregory (1941) found the perfect stage which he named *Sclerotinia narcissicola* and Buchwald (1949) transferred it to the genus *Botryotinia* Whetzel.

The smallest sclerotium-like structures are 100-125  $\mu$  in diameter and are referred to by McWhorter and Weiss (1932) as the small scale-speck fungus. Alecock (1924), Drayton (1927), Beaumont (1935), and Moore (1939) have referred to this fungus and to its appearance on the scales. It has been found also on hyacinth and snowdrop bulbs. No attempt has been made to name it, but from observations made on two occasions it seems probable that these structures are not true sclerotia but incompletely developed pycnidia which, at maturity, may develop a prominent beak.

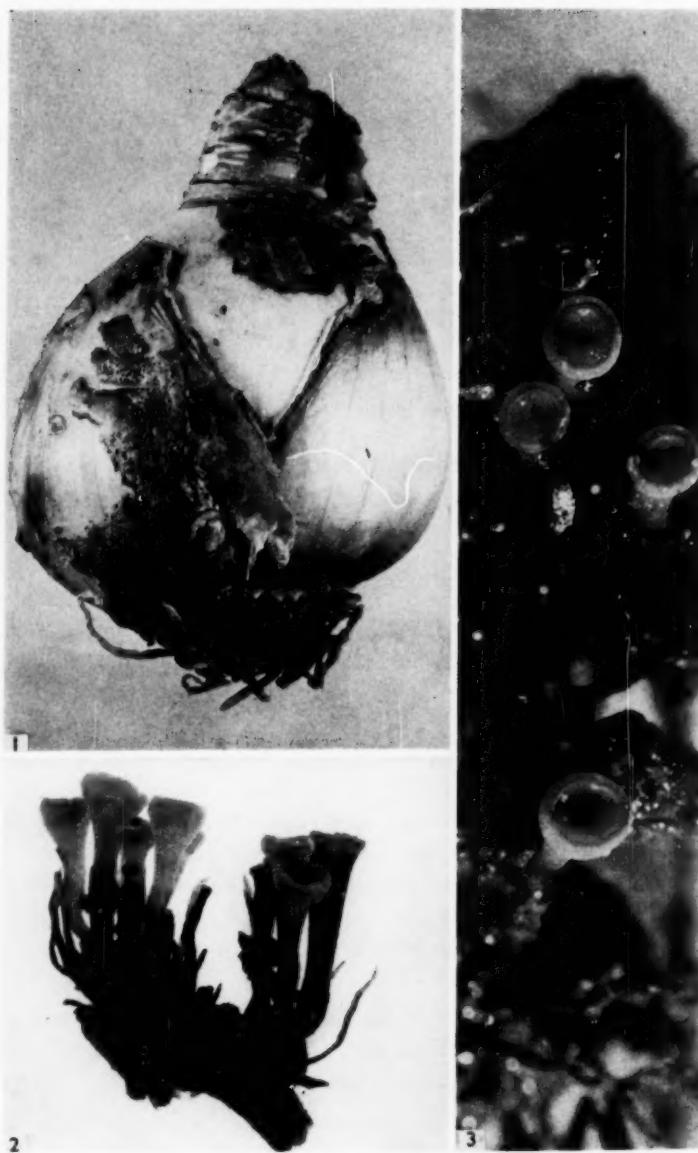
The third fungus, which produces sclerotia somewhat intermediate in size and which has been called the large scale-speck fungus by McWhorter and Weiss (1932), is the species under consideration in this paper.

The sclerotia are about 0.5-1.0 mm. in diameter, black, more or less circular, thin, flattened, and adhere firmly to the thin outer, papery scales. These bodies would be called "sclerotules" in the

<sup>1</sup> Contribution No. 1105 from the Division of Botany and Plant Pathology, Science Service, Ottawa, Canada.

<sup>2</sup> Assistant Chief, Division of Botany and Plant Pathology, Ottawa.

<sup>3</sup> Senior Mycologist, Central Laboratory, Ottawa.



FIGS. 1-3. 1. Narcissus bulb with sclerotules of *Stromatinia narcissi* on the outer scales,  $\times 1$ . Photo by A. J. Hicks. 2. Apothecia of *S. narcissi* from culture,  $\times 4$ . 3. The first apothecia of *S. narcissi* which were obtained in a pathogenicity trial. Photo by W. R. Fisher, Cornell University.

terminology adopted by Whetzel (1945). Their arrangement on the scales is most unusual in its regularity, for they develop in vertical rows which are approximately equidistant with the spacing in the rows (FIG. 1). Several series of inoculation experiments have failed to produce any disease and it is presumed that this fungus is wholly saprophytic. McWhorter and Weiss (1932) have noted that it is largely restricted to the bulbs of certain Bicolor varieties of narcissus, such as Victoria, Princeps, Spring Glory, and van Waveren's Giant. It has been reported from the Pacific Northwest of the United States and specimens have been found repeatedly in shipments of narcissus bulbs from Holland. Further specimens have been received from England and the United States Department of Agriculture Field Station, Babylon, Long Island, and two cultures were received from Dr. Freeman Weiss, Washington, D. C.

In a discussion of the two scale-speck fungi, Moore (1939) has pointed out the possible connection of one of these fungi with *Sclerotium ambiguum* Duby var. *narcissi* Sacc., described on the scales of narcissus bulbs in Palermo, Italy. The description by Saccardo (1910) is meagre, but, as far as it goes, it agrees with the appearance and size of the large scale-speck fungus.

#### DEVELOPMENT OF APOTHECIA

Isolations from the sclerotia on the bulb scales grow rapidly on potato-dextrose agar. They develop large numbers of sclerotia about 0.3-1.25 mm. in diameter which show no tendency to coalesce. The cultures have a musty odor similar to that noted in *Stromatinia gladioli* (Drayt.) Whetz.

In the earliest of the pathogenicity trials, sound narcissus bulbs were planted in six-inch pots containing soil mixed with a culture of the fungus on sterilized wheat grains and water. The pots were placed in a rooting cellar at a temperature of approximately 7° C. and kept moist. At the end of three months when these pots were being removed to the greenhouse, it was found that filiform structures similar to the receptive bodies in *Stromatinia gladioli* had developed all over the surface of the soil. These proved to be apothecial fundaments, for on standing in the greenhouse a very small percentage of these stalks developed into apothecia with discs

varying from 2.5-3.0 mm. in diameter (FIG. 3). A few single ascospore cultures were made from spores discharged by these apothecia and the fungus obtained was identical with the original isolate.

When an attempt was made to investigate the sexual mechanism in this species it was found that only one of the single ascospore cultures produced spermatia and that this culture failed to produce any receptive bodies. However, when the spermatia from this culture were used to fertilize the receptive bodies of the other cultures, a second crop of apothecia was obtained and from these a considerable number of single ascospores were isolated.

On the fifth day after planting these spores on potato-dextrose agar, it was evident that two types of mycelium were developing (FIG. 4). In one, the growth was rapid and the aerial mycelium distinctly floccose, while, in the other, growth was much slower and the aerial mycelium was silky, more appressed, and gave no evidence of tufting. In two to three weeks the difference between these two types of culture was even more striking, for the more rapidly growing one with floccose mycelium developed stromatic tissue and large numbers of the typical sclerotia, whereas the others developed within the medium a pseudostroma of large, black, intertwining hyphae, which macroscopically resembled a stroma, but lacked the consolidation of the true stroma developed by the other set of cultures. In addition they failed to produce any sclerotia, but water-soaked areas appeared over the surface, accompanied by a collapse of the aerial mycelium, and in these areas immense numbers of spermatia were found. In the other set of cultures no spermatia appeared. A microscopic examination of the mycelia of these two types of culture revealed distinct differences in the size of the hyphae. The type developing stromata and sclerotia has a great many hyphae 1.5-1.8  $\mu$  in diameter, a few 4.0-5.5  $\mu$  and occasionally some 7.5-8.0  $\mu$ . The other type has a few hyphae 2.5-3.5  $\mu$  in diameter but by far the majority are 6.0-7.5  $\mu$ . On the whole the hyphae of the cultures bearing spermatia are much larger than those in the cultures developing sclerotia and stromata.

These two sets of cultures were grown on sterilized wheat and water in Petri dishes and subjected to conditions favorable for the development of apothecia. Apothecial fundaments (FIG. 7) de-

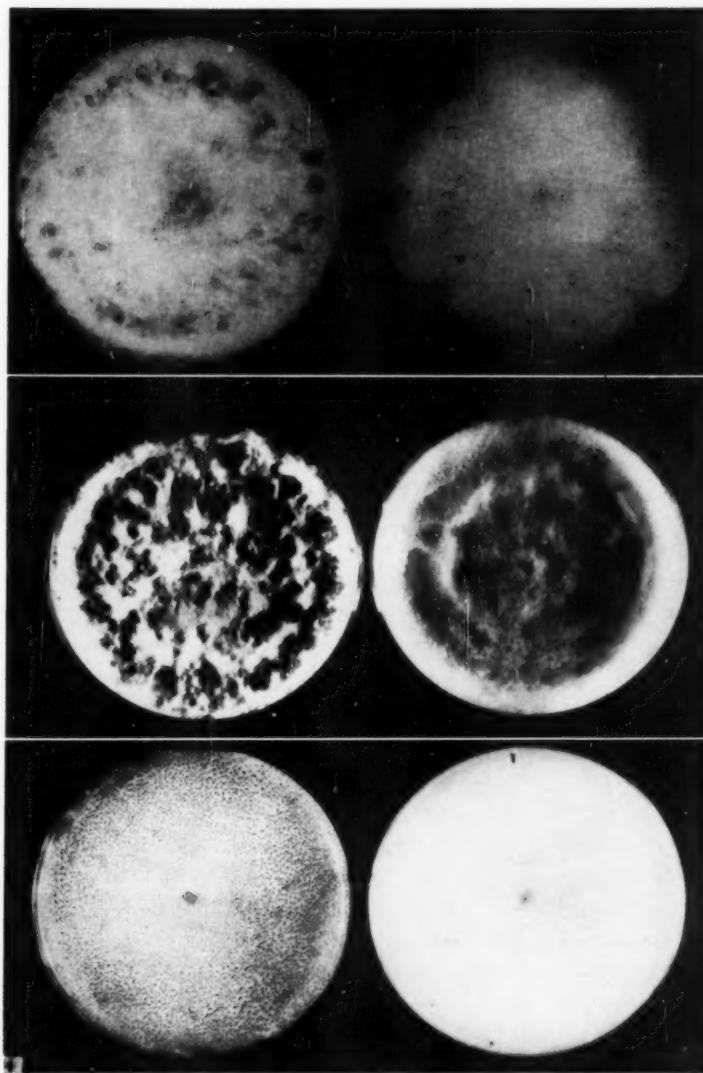


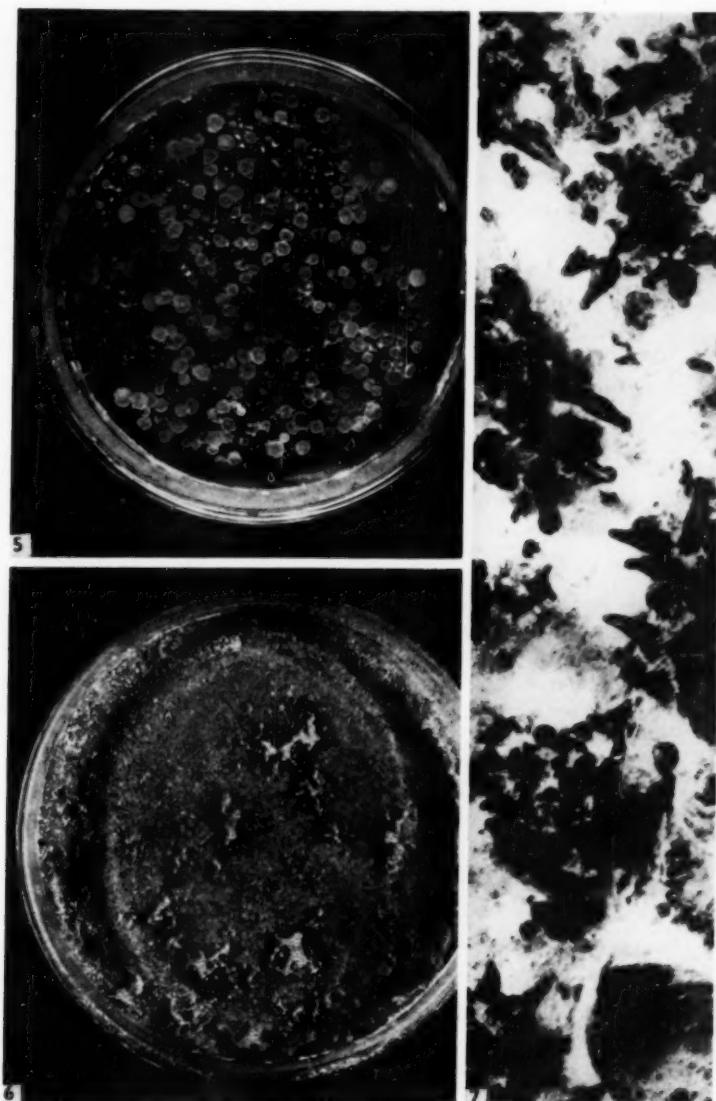
FIG. 4. Single ascospore cultures of *S. narcissi* on potato dextrose agar, male cultures on the right, female cultures on the left. Top, cultures about 4 days old; note floccose mycelium and faster growth rate in the female cultures. Center, cultures about 12 days old; black stroma is developing in the female culture and wet, collapsed areas appearing in the male culture. Bottom, reverse side of Petri dishes in 12-day-old culture showing sclerotules in the female. Photo by R. E. Fitzpatrick.

veloped from the stromata of the one set of cultures, whereas the other set continued to produce spermatia. By spermatizing the apothecial fundaments with spermatia from the other set of cultures, mature apothecia were obtained which were identical with the ones in the original culture series. Single ascospore isolations from these apothecia yielded the same two types of culture which are considered to be female and male respectively. A total of 83 random isolations of single ascospores from three generations of apothecia yielded 41 male and 42 female cultures.

The technique used to produce apothecia was essentially the same as reported previously, Drayton (1934b, 1937). Some variations in the schedule were tried and the most successful method for this species proved to be as follows. Cultures were grown on sterilized wheat grains at 14° C. in the dark about five weeks. At this time receptive bodies have started to develop from the stroma. This development was retarded by placing the dishes at 5° C. in the dark for two weeks. The entire culture, including the wheat grains, was then placed on moist quartz sand in culture dishes and spermatized. The male cultures were also grown on wheat and kept in the dark at 14° C. during the entire period. A suspension of the spermatia was prepared in sterilized soil extract and this suspension was added to moist, sandy, sterilized soil. The wet soil was then spread carefully over the surface of the thallus with a sterilized camel's hair brush, and over the edge of the thallus so as to make contact with the moist quartz sand. Following spermatization, dishes were placed at 0° C. for two weeks and then returned to 5° C. for ten days before being placed in the greenhouse. They were shaded from direct sunlight but exposed to north light, and apothecia matured in five to six weeks (FIGS. 5, 6).

The entire schedule from the time the fungus was first planted on wheat to the appearance of mature apothecia occupied about 4-5 months. This species appears to be sensitive to high temperatures and drying, and several series were ruined through mishaps, such as a refrigerator going out of order or the temperature in the greenhouse becoming too high. The most successful series were those that were planned in such a way that the dishes were brought into the greenhouse during January or February.

A number of attempts were made to isolate the eight spores from



FIGS. 5-7. 5. Culture dish with apothecia of *S. narcissi* from a fertilized culture. 6. Culture dish with unfertilized culture of *S. narcissi*. 7. Apothecial fundaments developing in a wheat plate culture,  $\times 8$  approx. Photo by R. E. Fitzpatrick.

one ascus. It was found impossible to dissect the spores from the ascus for the spores are small and hyaline and the ascus walls rather tough so that some of the spores invariably were lost. However, it was found possible to isolate the eight spores from one ascus after they had been discharged. The method was to embed the stalk of an apothecium in a small piece of plasticine which was then pressed on to the lid of a Petri dish containing potato-dextrose agar. The plasticine could be manipulated in such a way that the disc of the apothecium was suspended at a slight angle about 3-4 mm. over the surface of the agar. The lid was then rotated slowly and the apothecium was allowed to discharge spores on to the agar. By allowing it to remain in one position for short periods of 1-3 seconds, it was possible to find groups of eight spores, well isolated from other spores and obviously discharged from one ascus. By leaving the apothecium at a slight angle the spores were sufficiently scattered so that they could be picked up individually by means of a fine needle under the high power lens of the dissecting microscope, the illumination being a combination of sub-stage and surface lighting.

In this way several groups of eight spores from individual ascii were isolated, and in every instance four of the spores gave rise to male thalli and four of them gave rise to female thalli. The order of the spores in the ascus was not determined.

#### TECHNICAL DESCRIPTION

##### **Stromatinia narcissi** sp. nov.

*Sclerotinia narcissi* Whitehouse, nom. nud. Biol. Rev. 23: 432.  
1949.

*Sclerotium ambiguum* Duby var. *narcissi* Sacc. Ann. Myc. 8:  
347. 1910.

Apothecia singula vel fasciculata ex stromate, stipitata, 2.0-4.0-(10.0) mm. diam., 5-15 mm. alt., carnosso-coriacea, a profunde salviformibus subumbiliata, brunnea, disco olivascente, margine paullum incurvata; stipes paullulum angustatus inferne, imus subniger veratro-brunneus, gradatim superne pallescens; hypothecium stipesque prosenchymatica cum regione excipulari plectenchymatica munita; asci cylindrico-clavati, valde stipitati, 135-190  $\times$  (7)-8-10-(11)  $\mu$ , octospori; ascosporeae in primis hyalinae, ab ellipsoideis ellipsoideo-fusiformes, unicellulares, (8.0)-10.0-15.0-(18.0)  $\times$  (3.0)-4.0-5.5  $\mu$ ,

deinde pallide olivaceo brunnescentes, maturitate degenerant uniseptatae latiores que 5.5-7.0-(8.0)  $\mu$ ; paraphyses hyalinæ filiformes, 1.5-2.5  $\mu$  diam.; conidia nulla; spermatio hyalina, globosa 1.2-2.0  $\mu$  diam.; sclerotulae  $\pm$  circulares, compressæ, atrae 0.5-2.0 mm. diam.; ascospora singula culturam parit vel masculam vel foemina; mascula sericea, appressa, spermatio et pseudostromata generat; foemina floccosa, sclerotula, stromata, et corpuscula receptiva generat.

Hospes: tegumenta externa papyracea bulborum *Narcissi* et *Zephyranthis*.

Apothecia arising from the stroma singly or in clusters, stipitate, 2.0-4.0-(10) mm. in diameter, 5-15 mm. in height, tough-fleshy in consistency, slightly pubescent to nearly smooth, shallow cup-shaped to subumbilicate, becoming plane or sometimes convex-umbilicate in age, reddish-brown (Saccardo's Umber, Raw Umber, Buffy Brown, Auburn, or Prout's Brown<sup>4</sup>), in age the disc becoming somewhat olivaceous, the margin slightly incurved, paler than the hymenium, the stipe slightly tapering downwards, blackish or dark brown at the base, becoming paler upwards; tissue of the hypothecium prosenchymatous, composed of hyaline, closely interwoven hyphae about 3-5  $\mu$  in diameter, with an excipular zone composed of larger hyphae with irregular cells 4-10  $\mu$  in diameter and the walls somewhat gelatinized and the outer cells sometimes slightly darkened and often giving rise to short hairs about 3-4  $\mu$  in diameter, hyaline above but becoming brownish toward the base of the stipe; tissue of the stipe similar in structure, with the hyphae of the central medulla larger, 5-8  $\mu$  in diameter, more or less vertically parallel and more loosely arranged, the outer cells of the excipular zone becoming blackened, especially toward the base, and forming a rind-like layer, gradually merging into the stroma which is usually 0.5-1.0 mm. in diameter and similar in structure to the stipe; asci cylindric-clavate, tapering to a long, slender stalk, eight spored, 135-190  $\times$  (7.0)-8.0-10.0-(11.0)  $\mu$ ; ascospores at first hyaline, ellipsoid to ellipsoid-fusiform, sometimes slightly inequilateral, one-celled, irregularly uniseriate to partly biseriate, (8.0)-10.0-15.0-(18.0)  $\times$  (3.0)-4.0-5.5  $\mu$ , in age darkening to pale olive-brown, frequently becoming uniseptate, and becoming slightly broader, 5.5-7.0-(8.0)  $\mu$ ; paraphyses hyaline, filiform, septate, not branched, 1.5-2.5  $\mu$  in diameter, the tips rounded but not swollen; conidia lacking; spermatio hyaline, globose, uniguttulate, 1.2-2.0  $\mu$  in diameter, borne on flask-shaped phialides which may be aggregated to form spermodochia; sclerotules more or less circular, flattened, black, 0.5-2.0 mm. in diameter; single ascospores giving rise to either male or female cultures, the male cultures silky, appressed,

<sup>4</sup> Ridgway, Robert. Color Standards and Nomenclature. Washington. 1912.

producing spermatia and pseudostroma, the female cultures floccose, producing sclerotules, stroma, and receptive bodies.

Host: Outer papery scales of bulbs of *Narcissus* and *Zephyranthes*.

Type: Mycological Herbarium, Division of Botany and Plant Pathology, Science Service, Ottawa. No. 26023. Co-type material deposited in University of Toronto Herbarium; Farlow Herbarium; U. S. Dept. Agr. Herbarium; Kew Herbarium; Her-

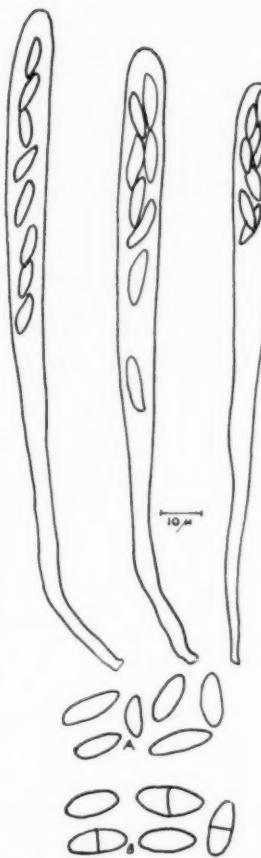


FIG. 8. Drawings of asci and ascospores, (a) ascospores freshly discharged from normal apothecia, (b) ascospores from overmature apothecia.

barium of the Museum of Natural History, Paris; Herbarium of the Institute of Systematic Botany, Uppsala.

One of the peculiarities of this species is the change in appearance of the ascospores when old. As far as we could determine the ascospores when discharged are always hyaline and one-celled (FIG. 8A), but examination of crushed mounts of over-mature apothecia disclosed many spores that were two-celled, slightly colored, and almost invariably somewhat broader than the hyaline, one-celled spores (FIG. 8B).

Although the usual host of this fungus is *Narcissus*, one specimen on *Zephyranthes* was received from Dr. Freeman Weiss, Washington, D. C. Isolations from the sclerotia yielded cultures that were typical of the female cultures of *S. narcissi*. Unfortunately these cultures were killed by a sulfur dioxide leak in the refrigerator in which they were stored before attempts were made to cross them with the male cultures of *S. narcissi*.

It might be observed that routine isolations made from sclerotia in nature always yield female cultures since only the females produce sclerotia.

#### TAXONOMY

This fungus on *Narcissus* is unquestionably congeneric with *Sclerotinia gladioli* Drayton (1934a) but is not regarded as congeneric with *S. sclerotiorum* (Lib.) deBary. The principal distinction is that in *S. sclerotiorum* and related species the apothecia arise directly from the tuberoid sclerotia, whereas in *S. gladioli* they arise from a stroma with female receptive structures developed on the stroma prior to fertilization, and the sclerotia are small and purely vegetative in function. Whetzel (1945) has called these small sclerotia "sclerotules," thus emphasizing a fundamental distinction between these bodies and sclerotia of the type found in *S. sclerotiorum*.

Whetzel (1945) transferred *S. gladioli* to the genus *Stromatinia* Boud. but the exact status of this genus has remained somewhat in doubt, as indicated by the accounts of Honey (1928) and Buchwald (1949). It is thus necessary to decide (a) the type species of *Stromatinia*, and (b) whether *S. gladioli* is congeneric with it.

The genus *Stromatinia* was erected by Boudier (1907) and de-

fined as including species in which the apothecia arise from a stroma. He did not designate any particular species as the type and under these circumstances it is permissible to choose any one as the type, but it is desirable to make the choice in such a way as to maintain stability in nomenclature, and it is preferable to follow the first author who does designate a type. Honey (1928), Nannfeldt (1932), and Whetzel (1945) have recognized *S. rapulum* (Bull.) Boud. as the type, but Buchwald (1949) has chosen *S. pseudotuberosa* (Rehm) Boud. Since it is now generally agreed that *S. rapulum* and *S. pseudotuberosa* are not congeneric, it is necessary to consider the nomenclatural consequences of recognizing one or the other as the type of *Stromatinia*.

*S. pseudotuberosa* is regarded by Buchwald (1949) as congeneric with *Ciboria caucus* (Rebent.) Fckl., the type of the genus *Ciboria*. We agree that *S. pseudotuberosa* is a *Ciboria*. The consequence of choosing this species as the type of *Stromatinia* is, therefore, that *Stromatinia* becomes a synonym of *Ciboria* and disappears as a generic name. Buchwald (1949) gave the name subgeneric rank under *Ciboria*, with *C. batschiana* (Zopf) Buchw. (= *C. pseudotuberosa* Rehm) as the type of the subgenus. In Buchwald's treatment *Stromatinia rapulum* and *S. gladioli* are placed in the section *Microsclerotiophorae* of the genus *Sclerotinia* with the type species *S. sclerotiorum* (Lib.) deBary. However, as noted above, from our studies of this group we are firmly of the opinion that *S. gladioli* is not congeneric with *S. sclerotiorum*; hence the recognition of *Ciboria pseudotuberosa* as the type of *Stromatinia* would necessitate the creation of a new genus for *S. gladioli* and related species.

On the other hand, if *S. rapulum* is recognized as the type and *S. gladioli* and *S. narcissi* can be shown to be congeneric with it, the genus *Stromatinia* is available for them.

In support of the choice of *Stromatinia rapulum* (Bull.) Boud. (= *Pesiza rapulum* Bull.) as the type of *Stromatinia*, it may be pointed out that it was the first species listed by Boudier (1907) and might be presumed to represent his idea of the type, that it has been already recognized as such by Honey (1928), Nannfeldt (1932), and Whetzel (1945). Its choice, therefore, renders un-

necessary the creation of a new genus for the species with apothecia arising from a stroma and producing sclerotules, and it provides an eminently suitable generic name for these species. Buchwald (1949) himself recognized that, if *Stromatinia* were to have generic status, *S. rapulum* should be the type and proposed that, in that case, his subgenus *Stromatinia* of *Ciboria* should be named instead *Pseudociboria*.

We are, therefore, of the opinion that *Stromatinia rapulum* should be accepted as the type of *Stromatinia*, but the problem still remains of determining whether *S. gladioli* and *S. narcissi* are congeneric with *S. rapulum*. This fungus was described as occurring on rhizomes of *Polygonatum*, but we have never collected any Sclerotiniaceae on this host or been able to study this species in culture. However, during the summer of 1950 the junior author had the opportunity of examining specimens labelled *Peziza rapulum* Bull. in the Persoon herbarium at the Rijksherbarium, Leiden, Holland, and specimens that Boudier had studied in the herbarium of the Museum of Natural History, Paris.

The Persoon specimen had been collected near Paris and consisted of two apothecia, 2 mm. and 10 mm. in diameter, rather deep cup-shaped, blackish brown, with the hymenium nearly black. The stipes had been broken off and there was no indication of the attachment. A mount showed abundant spores, hyaline, one-celled, ellipsoid to ellipsoid-fusiform, slightly inequilateral, uniseriate,  $10-16 \times 5.0-7.5 \mu$ . The asci were about  $8-10 \mu$  in diameter, but the length could not be accurately determined from this mount, probably about  $130-160 \mu$ .

In the herbarium at Paris there were several specimens of *S. rapulum* and they agreed in gross appearance with those in the Persoon herbarium. A mount from one showed asci and spores that also agreed with the Persoon specimens. There were also two drawings by Boudier, one showing three apothecia arising from a rhizome, the other showing five apothecia likewise. The specimen from which the drawing of the three apothecia was made, was clearly recognizable among the dried material preserved, and the apothecia were obviously arising from a thin stromatic tissue and not from sclerotia.

Apparently the fact that sclerotia were known to occur in *S. rapulum* caused Honey (1928) and Buchwald (1949) to doubt the desirability of maintaining *Stromatinia* as a distinct genus and was the reason why they placed the fungus in *Sclerotinia*. However, it is clear from the Boudier specimens that the apothecia arise from the stroma and not from the sclerotia.

As we have pointed out above, in *S. gladioli* and *S. narcissi* the apothecia also arise from the stroma and the sclerotia are purely vegetative in function. This is considered to be a fundamental difference worthy of generic rank. It is, therefore, concluded that *Stromatinia* is a valid genus, that *S. rapulum* should be recognized as the type, and that *S. gladioli* and *S. narcissi* can justifiably be regarded as congeneric with *S. rapulum*.

#### DISCUSSION

In its sexual behavior *S. narcissi* is unique, as far as is known, among the higher Ascomycetes and Basidiomycetes, with the exception of some of the Laboulbeniales. Species of the Sclerotiniaeae that have been investigated previously have shown two types of sexual behavior and *S. narcissi* now provides a third. These different types of behavior are of importance in relation to the question of mechanisms for bringing about outbreeding.

The biological advantages of outbreeding have been pointed out many times and in the plant kingdom various mechanisms and devices have been developed to bring this about. These mechanisms have been developed both in the haploid or gametophyte generation and in the diploid or sporophyte generation, and in describing them it is considered desirable to differentiate between the mechanisms in these respective generations.

One of the most effective mechanisms for bringing about outbreeding is the separation of the sexes so that male gametes are produced by one individual and female gametes by a different individual. This occurs both in the diploid generation in forms such as *Salix* spp., and in the haploid generation as in the seed plants and heterosporous Pteridophytes. In the diploid generation this condition is known as dioecism and species in which the male and female organs are borne on different individuals are said to be dioecious, whereas hermaphroditic species in which both male and

female gametes are produced on the same individual are said to be monoecious.

Blakeslee (1904) introduced the terms heterothallism and homothallism which he defined as corresponding in the haploid phase to the terms dioecism and monoecism in the diploid phase. Later (1906) he discussed these terms and listed, as examples of heterothallic forms, the liverwort *Marchantia polymorpha*, the heterosporous Pteridophytes, and the seed plants. It is, therefore, clear that he intended the term heterothallic to apply to haploid species in which the male and female sex organs were borne on different individuals. Furthermore with reference to homothallism he stated (1906, p. 175) "a morphological investigation may suffice to show that the male and female sex organs are borne on the same thallus and the form in question can then at once be classified as homothallic."

Therefore, if the terms heterothallic and homothallic are to be used in the sense of Blakeslee's definition, they can only be applied to species in which the male and female gametes are born on separate, haploid individuals.

In many diploid monoecious species outbreeding is encouraged by the development of other mechanisms. Mather (1940) has referred to some of these including mechanical devices such as special floral arrangements, physiological mechanisms such as protandry or protogyny, and genetic mechanisms such as incompatibility in *Nicotiana*. These methods may be more or less effective in promoting outbreeding and Mather has argued that, under certain conditions, incompatibility may be more efficient than unisexuality. However, none of these mechanisms has been confused in thought or terminology with dioecism.

In the haploid generation on the other hand, heterothallism as defined by Blakeslee has certainly been confused with other mechanisms. This is well illustrated by a consideration of the three types of sexual behavior found in the Sclerotiniaceae.

*Stromatinia narcissi* with distinct male and female thalli differing in gross appearance, growth rate, and diameter of hyphae, is an example of one type of behavior. This species is truly heterothallic in the sense of Blakeslee's definition.

*Sclerotinia sclerotiorum* (Lib.) deBary is an example of a second type of behavior. In this species each haploid thallus produces both male and female organs. The male cells are the spermatia borne on typical phialides and the female cells are borne in ascogonial coils beneath the rind of the sclerotium. When spermatia are placed on sclerotia from the same haploid thallus, apothecia are developed. This species is homothallic because both male and female sex organs are produced on the same haploid thallus and in addition it is self-fertile because the entire life history can be completed from a single haploid spore. No mechanism for promoting outbreeding appears to be present.

The third type of sexual behavior is illustrated by *Stromatinia gladioli* (Drayt.) Whetzel. Here, just as in *Sclerotinia sclerotiorum*, both male and female organs are borne on the same haploid thallus. The spermatia are produced on the usual phialides, but in this species the ascogonial coils develop in a stroma rather than in a sclerotium and become elevated in special structures that have been termed receptive bodies. Therefore, *S. gladioli* is also homothallic in the sense of Blakeslee's definition, but it is not self-fertile. Apothecia can only be produced by mating compatible isolates and compatibility appears to be determined by a single pair of genetic factors. It is important to note that reciprocal fertilization can take place between compatible isolates. Each haploid thallus not only bears both male and female organs but is capable of functioning either as a male or a female, when mated with a compatible isolate. *S. gladioli* is, therefore, homothallic and self-sterile and outbreeding is brought about by a mechanism of sterility factors. These might equally as well be called compatibility factors but will be referred to in this paper as sterility factors.

Although this mechanism is obviously quite different from that in *S. narcissi* it has also been called heterothallism. For example, Buller (1941) stated that in *S. gladioli* Drayton had discovered heterothallism in the Sclerotiniaceae, notwithstanding that Drayton himself (1934b) was careful to point out that the mechanism he had discovered was not heterothallism in Blakeslee's sense.

It is evident that other Ascomycetes which have been said to be heterothallic, such as *Neurospora sitophila* Shear & Dodge, *Ven-*

*turia inaequalis* (Cooke) Wint., *Ascobolus magnificus* Dodge, and many others, are similar to *S. gladioli* in their sexual behavior. They are homothallic in Blakeslee's sense and self-sterile with sexual compatibility determined by a mechanism of sterility factors.

A comparison of Buller's discussion of *S. gladioli* with his discussion of the Laboulbeniales reveals the confusion that arises from an attempt to apply the same term to two different mechanisms. With reference to the Laboulbeniales he states, "Here we have heterothallism expressed as sexual dimorphism, and the determination of sex takes place genotypically in the young ascus." However, according to Blakeslee's definition of the term this is heterothallism and not just an expression of heterothallism. We cannot logically use the term heterothallism for forms such as the sexually dimorphic Laboulbeniales and Phycomycetes, and *S. narcissi*, and also use the same term for forms such as *S. gladioli*, *Neurospora sitophila*, and *Ascobolus magnificus*.

In the so-called heterothallic rusts essentially the same situation occurs as in *S. gladioli*. Each haploid thallus gives rise to both spermatia and aelial primordia but the initiation of the dicaryon is dependent upon the mating of compatible thalli. Compatibility is determined by a mechanism of sterility factors and reciprocal diploidization is possible between compatible thalli. These species are, therefore, homothallic and self-sterile. It should be noted, however, that in the rusts the dicaryon can also be initiated by the fusion of hyphae of compatible thalli, but in *S. gladioli* fertilization cannot be accomplished by hyphal fusions between compatible isolates.

In the Ustilaginales, Hymenomycetes, and Gasteromycetes no morphological sex organs are present and diploidization is accomplished by means of cell fusions or hyphal fusions. It is significant, however, that when compatible isolates of a so-called heterothallic bipolar species are mated, reciprocal diploidization occurs. Each haploid thallus can function either as a male by passing nuclei to the other, or as a female by receiving nuclei from the other. The behaviour is similar to that of *Stromatinia gladioli* if we imagine no sex organs present in that species and if fertilization were effected as a result of hyphal fusions. It seems reasonable to con-

clude that mating ability in these forms is determined by a mechanism of sterility factors and not by a difference in sex. If it were the latter, we should expect that only one of the two thalli would become diploid, i.e., the male would fertilize the female. Brodie (1948) has described a phenomenon in *Cyathus stercoreus* that he has termed "unilateral diploidization" in which this evidently occurred in some matings. However, up to the present no basidiomycete is known in which definite male and female thalli have been distinguished comparable to the differences noted in *Stromatinia narcissi*. Thus, no basidiomycetes are heterothallic in Blakeslee's sense, but many are homothallic and self-sterile with compatibility determined by a mechanism of sterility factors.

Furthermore, in these groups the sterility factor mechanism becomes much more complicated and we have the appearance of the phenomena that have been termed tetrapolar sexuality and geographic races. Buller (1941) has discussed the theories proposed by Kniep and Hartmann to account for these phenomena, and it now appears that Kniep's theory is the one more generally accepted, namely that tetrapolar sexuality is the result of the occurrence of a second pair of sterility factors, and the geographic races are merely the expression of multiple allelomorphs of these two pairs of factors.

Mather (1942) and Whitehouse (1949a) have shown that the increase in complexity of the sterility factor mechanism leads to an increase in efficiency of outbreeding. For example, in a bipolar species 50% of the sister matings are fertile and 50% of the non-sister matings are fertile, but when multiple allelomorphs are present the fertility of non-sister matings may theoretically rise to 100%, thus doubling the proportion of fertility in non-sister as compared to sister matings. Similarly in a tetrapolar species 25% of the sister matings are fertile and 25% of the non-sister matings are fertile, but when multiple allelomorphs are present the fertility of non-sister matings may rise to 100% and the proportion of fertility in non-sister to sister matings is quadrupled.

It is, therefore, suggested that, in the fungi, the main line of evolution has depended on the sterility factor mechanism for bringing about outbreeding, whereas in the higher plants the main line of evolution has followed sexual differentiation in the haploid phase.

Whitehouse (1949a, b) has reviewed the literature in considerable detail and discussed the various mechanisms for bringing about outbreeding. He has clearly recognized the distinction between the sterility factor mechanism and separation of the sexes, but by altering Blakeslee's definition he has sought to include both mechanisms under the term heterothallism. He has distinguished them by applying the term morphological heterothallism to separation of sex and physiological heterothallism to the sterility factor mechanism. This is comparable to applying the term physiological dioecism to conditions such as protandry and protogyny in the flowering plants.

It might be questioned whether this alteration in the definition is justified. It is undoubtedly true that the fungus that originally suggested the idea to Blakeslee is not heterothallic in the sense in which he defined the term. The ability to mate was at first confused with sex in the absence of morphological sex organs and this confusion persisted for many years, even to the point where multiple sexes were postulated. However, we now know that a thallus may be bisexual and still be able to mate only with genetically compatible thalli. It is unfortunate that, probably because the idea was first suggested by a fungus, the term heterothallism has been taken up principally by the mycologists and consistently misapplied by them. Possibly the term in its misapplied sense is now too strongly entrenched in mycological literature to be dislodged and we shall have to justify the misapplication of the term by altering the definition to fit the misapplication. Nevertheless, it seems regrettable that this term should be altered in meaning to fit its misapplication, thus necessitating the use of a new term for its original meaning, for it was originally clearly defined as referring to a separation of sex in the haploid generation corresponding to dioecism in the diploid generation. It describes a phenomenon of fundamental biological importance in the plant kingdom, and it has also been properly applied by mycologists in certain groups of Phycomycetes and Laboulbeniales.

Whitehouse (1949a) has suggested that *S. narcissi* is a species in which the thallus is normally bisexual as in *Hypomyces solani* f. *cucurbitae* and that unisexual strains may have arisen by mutation. Hirsch (1947, 1949) has shown how this actually does occur in

*Hypomyces solani* f. *cucurbitae*. It is quite conceivable that the condition in *S. narcissi* may have arisen at some time from a homothallic form in some such way. The occurrence of a single species with unisexual thalli in a group in which the thalli of related species are bisexual would suggest that in this instance unisexuality was derived from bisexuality. However, in *S. narcissi* unisexuality appears to be well established as a character of the species and not merely a genetic aberration. Among the hundreds of cultures of *S. narcissi* examined, we have on two occasions observed a very few spermatia in old female cultures and on one occasion in a male culture observed some sclerotium-like bodies that proved to be soft and abnormal in texture. It is suggested, therefore, that it is the occasional bisexual strain that represents a genetic aberration in this normally unisexual species.

Whitehouse (1.c.) has also claimed that in *S. narcissi* there was evidence of a pair of sterility factors operating in addition to the sexual differentiation. It is again conceivable that, if unisexuality became established in a species that was originally bisexual with sterility factors represented by A and A<sub>1</sub>, we might find thalli that would be either ♂A, ♂A and ♀A<sub>1</sub>, ♀A<sub>1</sub>, or ♂A, ♂A<sub>1</sub>, and ♀A, ♀A<sub>1</sub>; hence all the males would not fertilize all the females. In a few attempts to cross all four males from one ascus with all four females from the same ascus, we found one ascus in which all the males fertilized all the females and two in which only two females were fertilized. If the condition suggested above existed, we should expect two of the males to fertilize two of the females and the other two males to fertilize the remaining two females. This did not happen but all four males fertilized the same two females and all four failed to fertilize the other two. Too few asci have been analysed to permit definite conclusions and this aspect of the problem needs further investigation, but the evidence does not support the theory that in *S. narcissi* we have both sexual differentiation and a two-allelomorph sterility factor mechanism.

In our view, therefore, *S. narcissi* is morphologically heterothallic in Whitehouse's terminology or truly heterothallic in Blakeslee's sense.

## ACKNOWLEDGMENTS

The writers gratefully acknowledge the assistance given by Dr. R. E. Fitzpatrick during the early phases of the investigation of this fungus and while he was a member of the staff of this laboratory. We are also indebted to him for some of the photographs. The photograph used in FIG. 3 was taken by Mr. W. R. Fisher, Department of Plant Pathology, Cornell University. The Latin diagnosis was prepared by Dr. B. Boivin of this laboratory.

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## THE EFFECT OF METHOD OF INOCULATION OF MEDIA ON SPORULATION OF *MELANCONIUM FULIGINEUM*<sup>1, 2</sup>

MARGARET B. TIMNICK,<sup>3</sup> H. L. BARNETT AND VIRGIL GREENE LILLY

(WITH 7 FIGURES)

The most common method of inoculating culture media for the growth of fungi in the laboratory is by the transfer of a bit of mycelium or a few spores to fresh medium. Agar media are usually inoculated at one point, from which the mycelium grows outward absorbing nutrients from the fresh medium as it penetrates new areas. Some fungi grown in this way produce abundant mycelium but fruiting and sporulation may be delayed until the mycelium has reached a certain age or stage of maturity, often after maximum weight is reached.

This characteristic is apparently more common among the fungi which produce their spores in definite fruit bodies, such as perithecia, pycnidia or acervuli. The Moniliaceous fungi more commonly produce conidia quickly on comparatively young mycelium. Sporulation usually begins near the inoculum and progresses radially. The conditions governing sporulation and the factors which lead to the early production of spores are not well understood.

During the course of an investigation into the factors affecting sporulation, it was discovered that the method of inoculating agar media determined to a large extent the rapidity of maximum sporulation of some fungi. *Melanconium fuligineum* was selected as the test organism in the studies reported here. A study of the nutritional factors affecting sporulation of this fungus has been reported in another paper by Timnick *et al.* (2).

<sup>1</sup> Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 438.

<sup>2</sup> This paper is based on work conducted for Camp Detrick, Maryland, under contract No. W-15-035-CE-167 with West Virginia University.

<sup>3</sup> Formerly research assistant, Department of Plant Pathology and Bacteriology.

## MATERIALS AND METHODS

The isolate of the fungus used in this investigation was obtained from a rotted grape at Morgantown, West Virginia. Other isolates were obtained but since they were all very similar only one was studied intensively. Petri dishes containing 25 ml. of agar medium were used. Since nutritional studies showed that the fungus sporulated most abundantly on a maltose-ammonium tartrate medium, this medium was used in the intensive studies on methods of inoculation. It had the following composition: maltose, 20 g.; ammonium tartrate, 2.8 g.;  $\text{KH}_2\text{PO}_4$ , 1 g.;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g.; thiamine, 50  $\mu\text{g}$ ; iron, 0.2 mg.; zinc, 0.2 mg.; manganese, 0.1 mg.; agar, 20 g.; distilled water to make one liter. The pH of the culture medium was adjusted to 5.0 before autoclaving.

Types of inoculum tested were spore suspensions in distilled water, discs of non-sporulating mycelium on agar and fragmented non-sporulating mycelium. The latter was prepared by adding a non-sporulating culture to 50 ml. of sterile distilled water and cutting for 30 seconds in a Waring Blender. Two general methods of inoculating agar media were used: (1) by placing the inoculum at one point at the center; (2) by flooding the entire surface with a suspension of spores or cut mycelium.

All cultures were incubated in a constant temperature room at 25° C. with alternating 12-hour periods of light and darkness.

Estimates of the abundance of sporulation were made in some cases by use of a haemocytometer. The material was prepared by placing the entire agar culture with a known quantity of water in a Waring Blender and cutting for one minute or more. This treatment fragmented the mycelium into small bits and did not harm the spores.

All experiments were repeated several times with similar results.

## EXPERIMENTAL RESULTS

When a suitable agar medium was inoculated with a bit of mycelium or a few spores placed at one point, the mycelium covered the area of a Petri dish within five or six days. A young culture is shown in FIG. 1. Sporulation began after about 11 to 14 days and reached a maximum after about 20 days. The spores were produced in definite large acervulus-like hyphal masses and col-

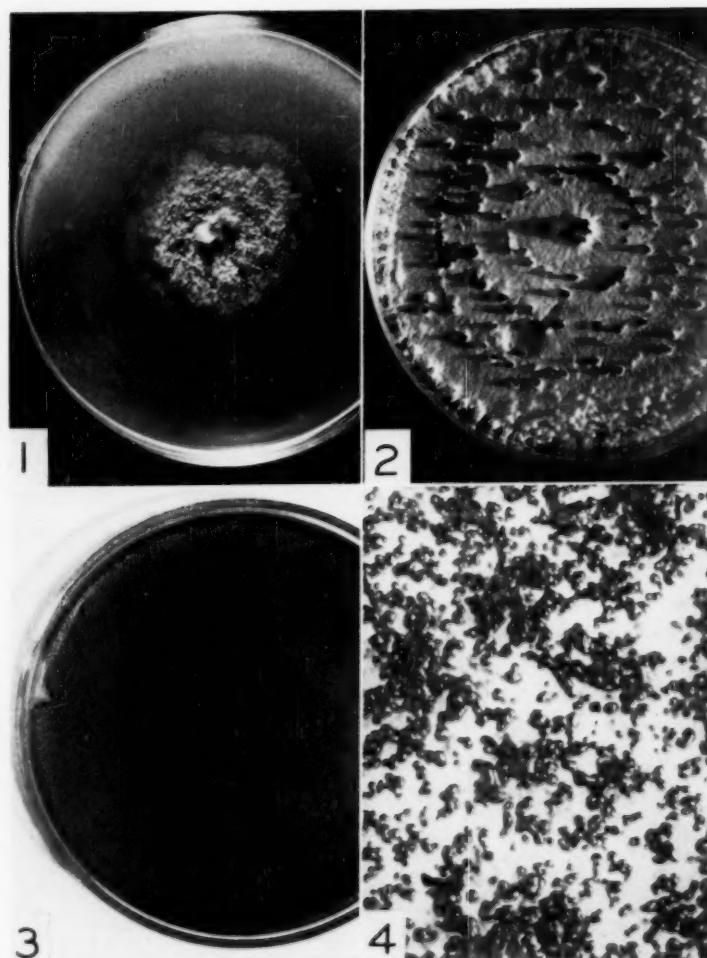
lected in dark droplets of various sizes (FIG. 2). Sometimes the spores were forced up through the compact mycelium and took the form of tendril-like cirri. Maximum sporulation under these conditions was estimated by haemocytometer counts as approximately 1.2 billion spores per Petri dish culture.

When a drop of spore suspension was placed at the center of an agar plate, spores were soon formed on the area covered by the inoculum. From this area, the mycelium then grew outward and produced spores in the same manner as when a bit of mycelium was used as inoculum. The rapid production of spores on the area covered by the drop of spore suspension suggested flooding the entire agar surface with spores.

A heavy suspension of spores (approximately 2.5 million per ml.) was then prepared in sterile distilled water and one milliliter of the suspension was used to inoculate each plate. The Petri dish was agitated so that the entire surface was covered. Under these conditions the production of spores began about 24 hours after inoculation and continued rapidly, the maximum being reached within four days. At this time nearly the entire surface was covered with shiny black cushions of spores (FIGS. 3 & 4). Little aerial mycelial growth was produced but microscopic examination revealed that the mycelium completely permeated the agar.

Repeated experiments showed that the concentration of spores in the suspension used as inoculum was an important factor affecting the time required for maximum sporulation of the subsequent cultures. A heavy spore suspension containing approximately 2.5 million spores per milliliter was then prepared. This suspension and different dilutions of it were used as inoculum. Maximum sporulation under any of these conditions, as determined by haemocytometer counts, was approximately 2.5 billion spores per culture. These cultures were arbitrarily given a sporulation rating of 5 and cultures producing fewer spores, as estimated visually, were rated by correspondingly lower numbers. These results are presented in TABLE I.

Petri dish cultures inoculated with the undiluted suspension of spores produced maximum sporulation within four days. After this time very few, if any, spores were produced. When fewer spores were used as inoculum the time required for maximum



FIGS. 1-4. 1. A 4-day-old culture started at one point on an agar plate. 2. A 25-day-old culture started at one point on agar. Note the dark masses of spores arising from acervuli and abundance of aerial mycelium. 3. A 4-day-old culture on an agar plate which was inoculated with a heavy spore suspension. Note the mass of spores nearly covering the surface and the absence of aerial mycelium. 4. An enlarged view of a portion of a culture like the one shown in Fig. 3. Note the separate cushions of spores.

sporulation was increased. In cultures inoculated with a highly diluted spore suspension numerous separate mycelia developed. The production of spores was delayed, being more nearly like that described above for plates inoculated at one point only. In general, the fewer the spores in the inoculum, the longer was the time required to begin sporulation and to reach maximum sporulation.

Non-sporulating mycelium was then finely cut in a Waring Blender and the suspension used as inoculum. The results were similar to those obtained when a suspension of spores was used, except that somewhat fewer spores were formed and the time required for maximum sporulation was somewhat longer.

TABLE I

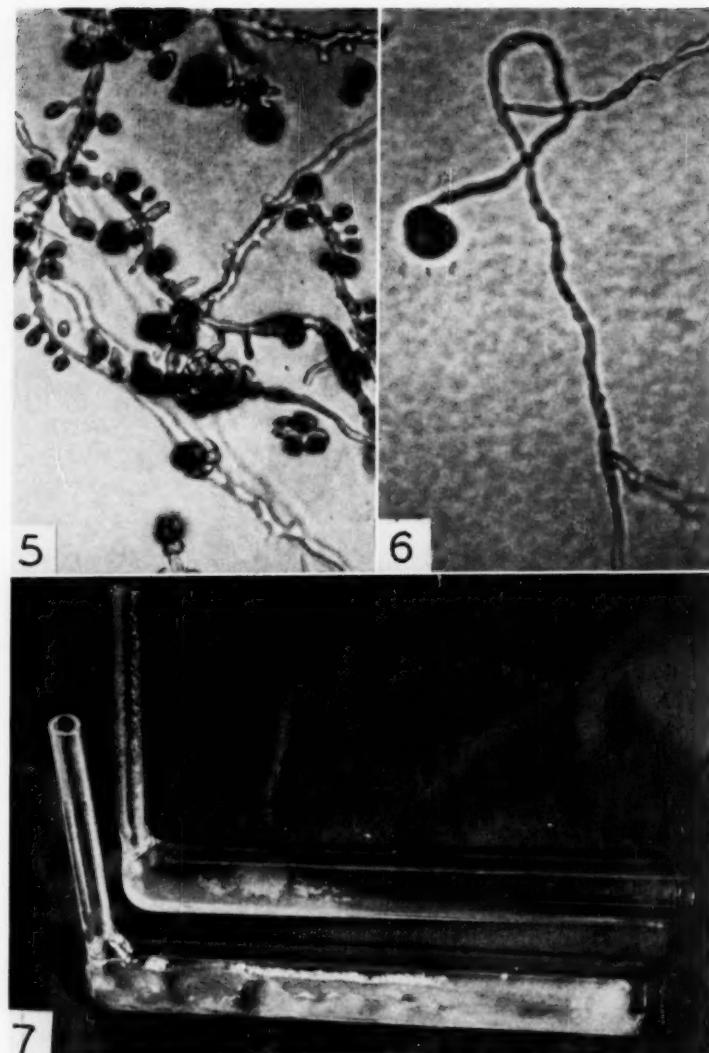
THE EFFECT OF CONCENTRATION OF SPORES IN THE SPORE SUSPENSION  
INOCULUM ON THE TIME AND ABUNDANCE OF SPORULATION  
OF THE SUBSEQUENT CULTURES

A sporulation rating of 5 equals approximately 2.5 billion spores per culture

Dilution of inoculum	Relative abundance of sporulation		
	4 days	8 days	16 days
Undiluted (2.5 million spores per ml.)	5	5	5
Diluted 1/10	4	4	5
Diluted 1/100	1	3	4
Diluted 1/1000	0	1	2
Diluted 1/10,000	0	1	2
Diluted 1/100,000	0	0	1

It was evident that the changes leading to sporulation of the cultures receiving heavy spore suspension inoculum must occur rapidly, soon after spore germination. Germination of the spores was then followed microscopically. The spores began to germinate after about 12 hours, sending out one or two germ tubes which soon branched, some of the branches turning downward into the medium. After 20 to 24 hours typical spores began to form at the tips of lateral peg-like structures on the germ tube and its branches (FIG. 5). This type of spore formation continued rapidly until cushion-like masses of spores were built up.

In contrast, when a dilute spore suspension was used as inoculum, the spores were widely separated. Germination began at about the same time and in the same manner as described above. In this case, however, the germ tubes continued to grow and branch,



FIGS. 5-7. 5. Photomicrograph showing the production of spores on germ tubes and young mycelia 36 hours after inoculation with heavy spore suspension. Compare with FIG. 6. 6. Photomicrograph of a single germinating spore on agar 36 hours after inoculation with a highly diluted spore suspension. Compare with FIG. 5. 7. Specially designed culture tubes in which the cultures were flooded continuously with distilled water (upper tube) or liquid medium (lower tube). Note the abundance of mycelium in the lower tube.

forming extensive mycelium with no early spore production (FIG. 6).

Several possible explanations for the phenomenon of rapid sporulation following a heavy spore suspension inoculum were considered. The possibility that a sudden change in pH of the medium was responsible was ruled out when it was discovered that media ranging from pH 4 to 8 gave nearly equally rapid sporulation. Likewise, no sporulation-inducing substance in or on the spores could be demonstrated. Furthermore, a heavy suspension of fragmented non-sporulating mycelium when used as inoculum resulted in sporulation nearly as rapid as when spores were used.

It was then believed that the sudden depletion of nutrients following the establishment of many growing points in some way brought about conditions, perhaps simple starvation, which favored sporulation. To test this hypothesis specially designed tubes were prepared and used as culture vessels (FIG. 7).<sup>4</sup> Agar media in these tubes were inoculated with a heavy spore suspension and a period of approximately 12 hours was allowed for the spores to germinate and become anchored to the agar. The tubes were then held in a nearly horizontal position by a clamp on a ring stand with the small side arm in a vertical position. A constant supply of sterile distilled water was introduced into the vertical arm at the rate of four to six drops per minute and allowed to pass over the culture and out of the lower end of the tube. In this way the surface of the culture was constantly bathed with the water, but not deeply covered. By this method the nutrients were slowly leached from the agar medium. Under these conditions numerous spores were produced within 24 hours after inoculation. Little mycelium was produced, even after continued incubation.

Sterile, liquid malt extract medium was passed over a duplicate set of cultures in the same way so that the nutrient supply was being replaced continuously. These cultures were examined at intervals of a few hours but no spores were present even after three days. At this time there was abundant mycelium in the tubes. Several repetitions of this experiment gave the same results.

The obvious conclusion from these experiments is that starvation

<sup>4</sup>This experiment was conducted by Mr. John Eichenmuller, graduate assistant in this department.

of the mycelium of *Melanconium fuligineum* is one of the more important factors involved in the rapid production of spores. Under the conditions of the experiments the cultures failed to sporulate as long as they received a continuous abundant supply of available nutrients.

The effects of flooding agar plates with a spore suspension have been tested with several other fungi, with varying degrees of success. Sufficient evidence is available to indicate that the time required for the beginning of spore production and for maximum sporulation of a number of fungi may be greatly reduced by this method.

#### DISCUSSION

It is characteristic of many fungi that they produce their spores only after vegetative growth has reached or passed the maximum. When cultures of *Melanconium fuligineum* are started on agar at one point the production of spores begins when the cultures are 11 to 14 days old. It is likely that the nutrient supply in the medium is nearly or completely exhausted at this time. The technique of flooding the agar medium with a concentrated suspension of spores shortens the minimum sporulation time to about 24 hours, maximum sporulation occurring within four days. Using this method the number of spores obtained after four days may be as great as one thousand times the number used as inoculum. Cultures started at one point only may produce approximately the same number of spores but require about 20 days to do so.

Since the diffusion of nutrients through agar is relatively slow, the exhaustion of nutrients from a particular area is accomplished chiefly by the advancing mycelium. As long as the growing mycelium finds a plentiful supply of nutrients vegetative growth is favored and the production of spores does not occur. Further evidence in support of this hypothesis was obtained when cultures leached by a continuous stream of water sporulated within 24 hours, while in other cultures which were continuously supplied with fresh liquid medium sporulation was delayed for three days or more, although vegetative growth was abundant. This is reminiscent of the experiments of Klebs (1) with *Saprolegnia mixta*, which grew and remained in the vegetative stage as long as it was supplied with nutrients but produced oospores when transferred to

pure water. We do not mean to imply that exhaustion of nutrients is the only condition which induces sporulation, but it is unquestionably an important factor for many fungi.

The multiplicity of growing points developing on media inoculated with a concentrated spore suspension limits the amount of nutrients available to each young mycelium. Apparently the depletion of the nutrients and the subsequent changes favoring sporulation occur so rapidly that the spores are borne directly on the germ tubes and young mycelium. One might consider this as early physiological maturity of these hyphae.

Preliminary experiments indicate that this phenomenon may be common to other fungi. This method of inoculating media may prove valuable for the rapid production of quantities of spores needed for use in pathogenicity studies, in fungicide tests, for teaching and other purposes.

#### SUMMARY

Cultures of *Melanconium fuligineum* growing on agar plates inoculated at one point by mycelium or a few spores required 11 to 14 days for sporulation, with the maximum production of approximately 1.2 billion spores per culture occurring after about 20 days. When a concentrated suspension of spores was used to flood the agar plates the formation of spores began within 24 hours and maximum sporulation of approximately 2.5 billion spores per culture occurred within 4 days. In the latter case the first spores were produced on the germ tubes and very young mycelium.

A hypothesis, based on the rapid depletion of nutrients by the multiplicity of growing points, is presented to explain the rapid production of spores following the flooding of agar plates with a heavy spore suspension.

DEPARTMENT OF PLANT PATHOLOGY AND BACTERIOLOGY,  
WEST VIRGINIA UNIVERSITY,  
MORGANTOWN, WEST VIRGINIA

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## NOTES AND BRIEF ARTICLES

### TWO PHALLOIDS FROM RHODE ISLAND

On August 24, 1951, Carl Wust, a student in bacteriology at Brown, collected a phalloid under a tree on a golf fairway at Warwick Neck, Rhode Island. There seems to be no doubt that it is *Pseudocolus javanicus* (Penzig) Lloyd [*Anthurus javanicus* (Penzig) G. H. Cunningham]. The collector reported that the carpophore was about 12 cm. tall, brilliant orange at the top to more yellow-orange at the base. It has 3 arms joined at the top and in the dried condition is 6.5 cm. tall without the volva (which was not collected), 2 cm. of which was stalk. The spores are elliptical to oblong-elliptical,  $4.5-6.1 \times 1.8-2 \mu$ , mostly  $5-5.5 \times 2 \mu$ , a little larger than the  $3-4.5 \times 1.5-2 \mu$  given by Cunningham (Gasteromycetes of Australia and New Zealand, 1944, p. 103).

In 1916, Sumstine (Mycologia 8: 183) reported *Colus Schellenbergiae* as a new species from Pittsburgh, Pennsylvania, with questions about *C. javanicus* Penz. Seaver found the same species at the New York Botanical Garden in 1928 and the two subsequent years, and concluded from a comparison with *C. javanicus* from Java that the two were identical. In 1949, Coker and Rebell (Mycologia 41: 280-282) reported *C. Shellenbergiae* or *Pseudocolus Schellenbergiae* (Sumstine) Johnson from Summit, New Jersey, and Ridley Park, Pennsylvania, with spores  $4-5(-5.5) \times 1.5-2 \mu$ . Cunningham (l.c.) includes *C. Schellenbergiae* and all but one of the species of *Pseudocolus* mentioned by Lloyd (Synopsis of the Known Phalloids, 1909, pp. 52-53) in *Anthurus javanicus*, since he does not recognize *Pseudocolus* Lloyd. Zeller (Mycologia 41: 46, 1949), on the other hand, does not recognize *Anthurus* as different from *Lysurus* but does accept *Pseudocolus*.

Another phalloid of somewhat wider distribution in the United States may also be reported from Rhode Island, even though it has been found in Massachusetts, because phalloids are not known to be of common occurrence in the Providence Plantations. This was

found in October, 1943, in a potato patch in Barrington. It is what has commonly passed as *Anthurus borealis* Burt or *Lysurus borealis* (Burt) P. Henn., more recently placed in *Lysurus Gardneri* Berkeley by Cunningham in agreement with Lloyd and accepted by Zeller.—WALTER H. SNELL AND ESTHER A. DICK.

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#### RESEARCH GRANTS OF THE MYCOLOGICAL SOCIETY OF AMERICA

The Mycological Society of America decided at its Council meeting of September, 1951, to make available to its members the annual income received from its sustaining members, to promote interest in the field of mycology, to further research and to serve as a special publication fund. The honorarium of the speaker for the Annual Lecture is to be paid from this fund, and the remainder is to be used for grants-in-aid. The sum of \$550.00 is available for 1951, and it can be assumed that approximately the same amount will be available for 1952. It is suggested that grants be limited to \$100. Application blanks may be obtained from Dr. Leland Shanor, Secretary-Treasurer, Department of Botany, University of Illinois, Urbana, Illinois. Applications, submitted in triplicate to the Chairman of the Research Grants Committee, should be in the hands of the Chairman by April 1, 1952.

##### The Committee:

Lee Bonar, University of California, Berkeley, California  
Grant D. Darker, Ben Venue Laboratories, Bedford, Ohio  
John N. Couch, Chairman, Committee on Research Grants, University of North Carolina, Chapel Hill, North Carolina

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#### LABORATORY TRAINING COURSES

A series of laboratory training courses is offered by the United States Public Health Service at the Communicable Disease Center, Chamblee, Georgia, throughout 1952. Of particular interest to those interested in medical mycology are: Laboratory Methods in Medical Mycology, Part 1, Cutaneous and Subcutaneous Fungi (March 31–April 11), Part 2, Systemic Fungi (April 14–25),

Laboratory Methods in Medical Mycology (Nov. 17-21) and Laboratory Methods in the Study of Pulmonary Mycoses (Nov. 24-Dec. 12).

Information and application forms should be requested from Chief, Laboratory Training Services, Communicable Disease Center, P. O. Box 185, Chamblee, Georgia.

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We regret to announce that Professor H. S. Jackson, Charter Member of the Mycological Society of America and President in 1934, died suddenly at his home in Toronto on December 17, last.

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## REVIEWS

**PHYSIOLOGY OF THE FUNGI**, by Virgil Greene Lilly and Horace L. Barnett. xii + 464 p., 81 figs. McGraw-Hill Book Company, Inc., New York, Toronto, London. 1951. Price \$7.50.

Lilly and Barnett, drawing freely on their own work, have written an introductory text on fungus physiology for students of mycology. They emphasize the need for experimental study of pure cultures and teach the student how to perform such experiments. The increasing recognition by mycologists that knowledge of physiology is necessary in all mycological studies will ensure a market for this book. Lilly and Barnett's discussion of morphologic changes effected by environmental conditions and examples of morphologic variability in the progeny of a single organism under uniform conditions should convince the last holdouts of the dangers of exsiccated mycology.

The authors give sound advice on methods for the study of fungi in pure culture, and devote considerable space to the biochemical background necessary for understanding the techniques. The elementary discussions of pH and enzyme action are necessary for the intended audience with mycological rather than physiological background. The authors are obviously widely read in biochemistry. If their reading seems to have been somewhat uncritical, one should remember that it is difficult to be expert in more than one field. Such defects as the confused and largely untenable discussion of "primary" vs. "secondary" amino acids, the acceptance of reports of nitrogen fixation by fungi and of the work of Nord and Mull on pathways of carbohydrate utilization (both ably criticized by Jackson Foster in "Chemical Activities of Fungi," 1949) will make little practical difference to students of non-biochemical aspects of mycology. The student of fungus biochemistry will continue to use Jackson Foster's "Chemical Activities of Fungi," appropriately supplemented.

The practical aspects of making media are discussed in a cogent fashion not equalled by any available text. Lilly and Barnett point out the fallacy of "chemical purity," the necessity for reporting the exact compounds used, the meaning of ways of expressing concentration, the importance of impurities in agar and cotton, and the deleterious effects of autoclaving. Insufficient appreciation of these points has been displayed by too many scientists who ought to have known better. The major point overlooked is the necessity for metal-complexing agents in liquid media which was demonstrated by L. Fries in 1945 and was recognized considerably earlier by workers who supplied iron as citrate. (See Fries, L. 1945. *Arkiv för Botanik* 32 A: 1-8.)

The association of fungi in nature and experimental studies of associations are also emphasized throughout the book, with figures showing symbiosis and antagonism under controlled conditions. The authors are not under the impression that unifungal pure cultures represent "natural" conditions. The chapters dealing with growth, factors affecting sporulation, spore discharge and dissemination are also excellent, though the effects of hormones and of genetic factors in reproduction might have been more fully discussed. One might wish also that the ecologic niches of *groups* of fungi, in terms of temperature preferences and the use of economically (*sensu latu*) important substrates, had been more fully discussed. Parasitism (and fungicides) receive considerable space and the groups of "obligate parasites" are mentioned. But while cellulose destroying fungi are named, the considerable literature on cellulolytic fungi as systematic groups has not been adequately used. Chitin and keratin are not mentioned as sources of carbon and energy.

Historical justice at least demands correct citations: the name of Burkholder, in whose laboratory chloromycetin was discovered, should not have been omitted from the list of authors of the paper announcing the discovery. But, in spite of other minor inaccuracies (*Thraustotheca clavata* has never been shown to require biotin), Lilly and Barnett have written the best text available for elementary instruction in fungus physiology.—HELEN SIMPSON VISHNIAC.

LES URÉDINÉES, by A. L. Guyot. Encyclopédie Mycologique, Vol. VIII, Tome I, Genre *Uromyces*. 438 pp., 83 figs. 1938. *Ibid.*, Vol. XV, Tome II. Genre *Uromyces*. 331 pp., 72 figs. 1951. Paul Lechevalier, Paris.

These two volumes represent the first issues of a projected taxonomic revision of the Uredinales of Europe and adjacent western Asia and northern Africa. The volumes will be most useful to persons primarily interested in the rusts of the areas mentioned. However, since the extra-European species are tabulated by geographical regions the result is essentially a synopsis of all of the species of *Uromyces*.

Guyot divides the genus, on the basis of the teliospores, into the following six sections: *tenues*-, *crassi*-, *papillati*-, *verrucosi*-, *angulati*-, and *coronati*-*Uromyces*. The species are grouped, firstly, according to the families of the hosts, and secondly, according to the above sections. Descriptions and illustrations in the form of line drawings are provided for species occurring in the European area and adequate, or sometimes exhaustive, discussion is appended. Several useful tabular comparisons of species together with distribution maps are employed. The work is well illustrated but one misses the usual determinative keys to species. There is a bibliography of 752 titles. Host and fungus indexes are also provided.

Tome I contains the species having telia on the Gramineae, Cyperaceae, Juncaceae, Ranunculaceae, Polygonaceae, Umbelliferae, and Campanulaceae. Tome II treats of the species on the Liliaceae, Amaryllidaceae, Iridaceae, Chenopodiaceae, Plumbaginaceae, Compositae, Rosaceae, Geraniaceae, Primulaceae, Scrophulariaceae, and Valerianaceae.

In general the work is commendable, but Guyot's inclusion of the many species of *Uromyces* on the Rosaceae will not meet with favor, since all of them legitimately belong in other genera. Apparently paper presented no problem since many pages, because of the spacing of paragraphs or the uneconomical groupings of figures, appear startlingly unoccupied.—GEORGE B. CUMMINS.

PUFFBALLS AND THEIR ALLIES IN MICHIGAN, by Alexander H. Smith. viii + 131 pp., 43 plates. The University of Michigan Press, Ann Arbor. 1950. Price \$3.00.

This is a welcome addition to the general treatment of a group of fungi, many of which are among the commonest and most conspicuous forms and, as such, of interest to the amateur and general collector as well as to the specialist. A brief introduction discusses methods of study, the nature of the gasteromycete fructification, and edibility. Classification follows Zeller's arrangement of 1949 and keys are provided leading to 10 orders, 29 families and 91 genera. Less than 90 species are described in detail as known or expected to occur in Michigan, but it is emphasized that careful collecting will surely reveal species and genera at present unknown from the State, and, as such are discovered, it is hoped that the keys will permit assignment at least to genera. Some 60 species and varieties are illustrated by excellent photographs, admirably selected for their purpose and well reproduced. In all cases, names are applied which the author believes are valid under existing rules. In a few instances references to more familiar names as synonyms might have proved helpful. The reviewer is glad to see *Astraeus* recognized, since, despite its superficial resemblance to *Gastrum*, it seems (to him) clearly distinct. He would question the assignment of *Secotium agaricoides* to that genus rather than to *Endoptychum*, where the key character would place it.—G. W. M.

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THE MOLDS AND MAN. AN INTRODUCTION TO THE FUNGI, by Clyde M. Christensen. viii + 244 pp. University of Minnesota Press, Minneapolis. 1951. Price \$4.00.

It has been said that the first requirement of sound scholarship is that its results must be presented in unreadable form. The present book fails to measure up to that requirement. It is absorbing, stimulating, amusing, sometimes irritating—all probably facets reflecting the same underlying quality. In its ten brief chapters it discusses what fungi are, how they reproduce and spread, their partnerships with plants and animals, their role as

parasites, their importance as agents of deterioration and their industrial exploitation. An eleventh chapter constitutes an appendix summarizing their classification.

The dust cover states that the book will serve as a basic text for students of mycology. This may be questioned. Mycology has become so extensive that a much more elaborate framework is needed. The real merit of Christensen's book is that it will clothe the dry bones of such a framework with very lively flesh. It will be invaluable as supplementary reading for classes in mycology and, more than that, it should appeal to many others who would like to know more about the fungi and who will find here a surprisingly large amount of information presented in vivid and colorful phraseology.

Naturally, a few errors of fact and some questionable opinions have crept in; none, so far as I have noted, serious enough to mention. The author implies in his preface that he has had a lot of fun studying the fungi. No one can read the book and doubt it. If it receives the circulation it deserves, many more should share that pleasure.—G. W. M.



## MANUSCRIPT

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